



IMPERIAL AGRICULTURAL
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16425

MGIPC—S4—III.1.93—22.8.45—5,000.

JOURNAL OF AGRICULTURAL RESEARCH

VOLUME 60

JANUARY 1-JULY 15, 1940



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WITH THE COOPERATION OF THE ASSOCIATION OF
LAND-GRANT COLLEGES AND UNIVERSITIES

UNITED STATES
GOVERNMENT PRINTING OFFICE
WASHINGTON : 1941

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Published on the 1st and 15th of each month. This volume will consist of 12 numbers and the contents and index.

Subscription price:

Entire Journal: Domestic, \$3.25 a year (2 volumes)

Foreign, \$4.75 a year (2 volumes)

Single numbers: Domestic, 15 cents

Foreign, 20 cents

Articles appearing in the Journal are printed separately and can be obtained by purchase at 5 cents a copy domestic; 8 cents foreign. If separates are desired in quantity, they should be ordered at the time the manuscript is sent to the printer. Address all correspondence regarding subscriptions and purchase of numbers and separates to the Superintendent of Documents, Government Printing Office, Washington, D. C.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 60

WASHINGTON, D. C.

JANUARY 7-JULY 15, 1940

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AUTHORS' ERRATA AND EMENDATION

Page 4, fourth line from bottom, " ρ' " should be " ρ' ."

Page 6, last line should read $S(y - Fy\xi'_1)(\rho'_0 - E\rho'_0\xi'_1) = S(y\rho'_0) - (Sy)(S\rho'_0)/n - S(\xi'_1y)S(\xi'_1\rho'_0)/S(\xi'_1)^2$.

Page 7, line 7, " $S(\xi'_1)^2$ " should be " $S(\xi'_1)^2$."

Page 10, table 6, second column, first line, " -0.00289563 " should be " $+0.00289563$."

Page 12, line 3, "for -23 to $+23$ " should be "for $n = 24$."

Page 41, sixteenth line from bottom "*Sisymbrium*" should be "*Sisymbrium*," and seventeenth line from bottom "*trudentata*" should be "*tridentata*."

Page 177, lines 6 to 16 from bottom, formula for media should have appeared as part of footnote 4.

Page 318, line 27, "reproduce" should be "produce."

Page 324, footnote 1, insert "or arsenic" between the words "lead" and "determination."

Page 329, reference 8a should also include the name of J. L. St. John.

Page 448, table 7, line 3 of data, column 4, minus sign should be a plus sign for pea viruses 2A, 2B, 2C under Perfection.

Page 636, table 3, last column, line 7 of figures, "59." should be "59.5," and in footnote 1, first line, insert the word "less" between "with" and "than."

Page 665, table 7, column 8, under "Adults tested by isolation," "1763" should be "1765."

Page 674, table 13, column 6, next to last line, "B" should be "3B."

Page 739, first paragraph, last line, the "or" should precede "American" in line above.

Page 744, line 7 of legend, "A,a" should be "A,b."

Page 778, seventh line from bottom, "music acid" should be "mucic acid."

Page 841, second line from bottom of text, insert "upon" between "unchanged" and "standing."

Page 847, fourth line from bottom, the name "Sweet" should be "Swett."

Page 852, in literature cited No. 1, "19" should be "1940"; ampersand should be "of"; add 128:355-371, illus.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 60

WASHINGTON, D. C., JANUARY 1, 1940

No. 1

EFFECT OF THE AMOUNT AND DISTRIBUTION OF RAINFALL AND EVAPORATION DURING THE GROWING SEASON ON YIELDS OF CORN AND SPRING WHEAT¹

By FLOYD E. DAVIS and J. E. PALLESEN, *assistant agricultural statisticians, Agricultural Marketing Service, United States Department of Agriculture*²

INTRODUCTION

The relation of weather to crop yields has been the subject of a large number of investigations in this country and abroad (*4*).³ In general, the usual correlation method of approach, which employs State yields and monthly weather data, has failed to provide a reliable regression equation for forecasting purposes, although instances may be cited in which it has yielded valuable information. These studies serve to emphasize the complexity of the relationships involved.

Yields of most crops are dependent to a large extent upon the weather during the growing season. In the present work it was desired to ascertain the influence on yield of certain weather factors at different stages during the season, and to determine the critical period of the growing season as regards these factors.

In studying the effect of rainfall on plot yields of winter wheat, Fisher (*1*) conceived the idea that rainfall occurring at any time during the growing season has an effect on final yield which can be expressed as $a(t)r(t)dt$, where $r(t)dt$ is the rainfall occurring during the interval t to $t+dt$, and $a(t)$ is a function of time expressing the physiological relation between amount of rainfall at time t and final yield. On this basis, the estimated yield, the rainfall during the season being known, will be

$$\bar{w} = c + \int_0^T a(t)r(t)dt.$$

This expression simply indicates that the estimate of yield is the product (rainfall) \times (the yield functions $a(t)$) summed over the entire season. It is clear that $a(t)$ will in general be a continuous function and one that changes relatively slowly. It may be expected, therefore, that it can be approximated with reasonable accuracy with a few terms of a polynomial in t . As a device for evaluating $a(t)$, Fisher used a set of orthogonal polynomials.

As pointed out by Fisher, this approach assumes that the effect of rain at any time is independent of the amount of rain falling at any other time. Nevertheless, relationships learned in this manner can be of considerable value in an understanding of the influence of weather factors on crop yields at different stages during the season.

¹ Received for publication July 6, 1939.

² The authors wish to express their appreciation to G. W. Snedecor, W. G. Cochran, and C. P. Winsor for helpful advice, suggestions, and criticisms. Appreciation is also expressed to C. F. Sarle and A. J. King, under whose supervision the study here reported was made.

³ Italic numbers in parentheses refer to Literature Cited, p. 22.

Such knowledge is of value to crop forecasters even though the deviations from regression due to other factors may be large.

* The primary purpose of this paper is to show the results of applying Fisher's technique to a study of weather factors and experiment station yields of corn and spring wheat. In view of the complicated nature of this technique, however, the paper is also designed to give the successive stages in the handling of the data. Improved methods of handling the computational work are also set forth. For the general method and theory one can profitably refer to Fisher's original paper, particularly to section 3. x

Data regarding annual plot yields for a few crops were made available to the Bureau of Agricultural Economics by a number of agricultural experiment stations throughout the country so that relationships between weather factors and yields might be studied. The method used in this paper is one of several that are being employed in these studies. The selection of the particular crops and of the yield series presented in this report was made on the basis of the opportunity they afforded to introduce the use of the present technique to crops and to weather factors not previously studied and to bring out certain refinements in the techniques. One of the first considerations in relating weather to yields is that a sufficiently long series of records be available. Fisher's analysis dealt with 60 years of yields, but nowhere in this country is there any series of continuous plot yields of that length. However, many series are of sufficient length to justify analysis by this technique.

DATA USED

Corn (*zea mays*) yields from plots at the Ohio Agricultural Experiment Station at Wooster, Ohio, and spring wheat (*Triticum aestivum*) yields from the Dry Land Experiment Station at Dickinson, N. Dak., were used in this study.

(Since 1894 at Wooster a series of plots has been cropped continuously to corn, with the more important weather factors observed concurrently. This series includes several treatments. The plots are located on Wooster silt loam and are slightly rolling. Plot 5, which has received a moderate treatment of manure each year (2½ tons per acre), has been used in this study. The use of lime on these soils was begun in 1905, and the yields from the date the liming practice was started have been used, giving a 32-year period (1905-36) with which to work.)

The yield data for spring wheat were obtained from the original records of the Division of Dry Land Agriculture, Bureau of Plant Industry, United States Department of Agriculture.⁴ Two plots receiving no fertilizer treatment have been cropped to wheat continuously since 1908. The spring wheat yields used are the averages of these two plots. (In 1912 hail destroyed the crop; consequently, no records for that year are included in the calculations.)

The meteorological data were obtained from the station records. Those used in this report are daily rainfall figures for the season at Wooster and daily rainfall and evaporation figures for the season at Dickinson. Before this study was begun these records had been drawn off and compiled into 5-day totals throughout the year.

⁴ These records were obtained through the courtesy of J. S. Cole.

Since the winter and spring rainfall is nearly always adequate at Wooster, the decision was made to investigate the effect of rainfall for a period of 120 days beginning a few days before planting time. The average date of planting was found to be about May 13. The approximate date of planting was ascertained for each year and the 5-day period in which this date occurred was marked on the rainfall tabulation. Then the third 5-day period previous to this was marked and was considered the beginning of the 120-day season for that year. Thus, the season of 120 days was different for each year, depending on the date of planting. Since the rainfall for the 120 days is compiled into totals of 5 days each; 24 such totals represent the season.

For spring wheat at Dickinson, the average date of seeding is about April 16 and the average date of harvesting August 5, with a mean interval of around 111 days. Here, as in the case of corn, a period of 120 days would seem adequate, and since evaporation data were available from April 1 of each year, it was decided to consider the crop season as beginning on that date and continuing for 120 days.

ANALYSIS OF DATA

METHOD OF ANALYSIS

In a direct attack on the relationship of a large number of independent weather variates (twenty-four 5-day totals of rainfall for the season) with yield, one would ordinarily set up equations in 24 unknowns $\alpha(1), \alpha(2), \dots, \alpha(24)$. The solution for these 24 regression coefficients directly would involve a tremendous amount of arithmetical work. Furthermore, with data for only 20 to 40 years, the high proportion of independent variables to observations would make it difficult to establish the significance of the regression coefficients. On physiological grounds it appears valid to assume that a coefficient of regression of yield on rainfall in one 5-day period would not differ widely from one in adjacent 5-day periods. Dropping the concept of a particular regression coefficient for a particular 5-day period, we can conceive a relation between yield and additional rainfall that changes slowly as we go from one short period of time to another. Such a function changing continuously with time is precisely what is to be represented by the function $a(t)$. (Looking upon $a(t)$ as a slowly changing function we assume that it can be represented by a polynomial and hence by a set of orthogonal polynomials.

The problem is now to evaluate the function a in equation

$$\bar{W} = C + \int_0^T a r dt \quad (1)$$

where a , as a continuous function, can be adequately represented by relatively few terms of a polynomial of the form

$$a = \alpha_0 T_0 + \alpha_1 T_1 + \alpha_2 T_2 + \dots \quad (2)$$

the T 's being the orthogonal functions of time. If (2) is substituted in (1) we get

$$\begin{aligned} \bar{W} &= C + \int_0^T (\alpha_0 T_0 + \alpha_1 T_1 + \dots) r dt \\ &= C + \alpha_0 \int_0^T T_0 r dt + \alpha_1 \int_0^T T_1 r dt + \dots \end{aligned} \quad (3)$$

Now the quantities $\int_0^T T_r dt$ are those one would get if orthogonal polynomials were fitted to the sequence of rainfall each year. From actual rainfall data in those years for which crop yields are available, one can calculate the quantities $\int_0^T T_r dt$. Placing these quantities in equation (3), the coefficients $\alpha_0, \alpha_1, \dots$ can be computed by multiple regression methods. Substituting the calculated partial regression coefficients in equation (2), we can evaluate the function α and represent at any time during the season the effect on the crop of an additional inch of rainfall.

In the application of his scheme, Fisher expressed a polynomial fitted to the sequence of rainfall in each year, as mentioned above, by

$$\rho_0 T_0 + \rho_1 T_1 \dots \quad (4)$$

in which ρ_i represents the quantities $\int_0^T T_r dt$ and the T 's are orthogonal and normalized functions of the time, that is,

$$\begin{aligned} \int_0^T T_r T_s dt &= 0 \quad (r \neq s) \\ \int_0^T T_r^2 dt &= 1 \end{aligned}$$

The values ρ_i may be calculated thus:

$$\rho_i = S(r T_i)$$

where r equals rainfall during a 5-day period and the summation extends over the given number of periods, noting that the integrations have been replaced by the summations.

But the numerical values are awkward to handle, and it is simpler arithmetically to use polynomials ξ_i' which are orthogonal but not normalized, and the values of which are integers. The polynomials are those tabulated by Fisher and Yates (3) and are related to those of Fisher by

$$\xi_i' = T_i \sqrt{S(\xi_i'^2)}$$

Employing the polynomials ξ_i' instead of the polynomials T_i , a set of coefficients ρ_i' is calculated that are related to the coefficients used by Fisher. The process of calculations

$$\rho_i' = S(r \xi_i')$$

is similar to that given above for ρ_i . The amount and distribution of rainfall is now expressed as a function of time by

$$\frac{\rho_0' \xi_0'}{S(\xi_0'^2)} + \frac{\rho_1' \xi_1'}{S(\xi_1'^2)} + \dots \quad (5)$$

The quantities here are related to those in Fisher's expression (equation (4)) by the equations

$$\xi_i' = T_i \sqrt{S(\xi_i'^2)}$$

$$\rho_i' = \rho_i \sqrt{S(\xi_i'^2)}$$

Since the values $S(\xi_i'^2)$ are given in the tables, Fisher's coefficients ρ_i could easily be calculated, but it is to be shown here that these numerous divisions may be avoided.

If the regression of yield on the coefficients $\rho_0', \rho_1', \rho_2', \dots$ is calculated, the partial regression coefficients obtained may be designated as $\alpha_0', \alpha_1', \alpha_2', \dots$. These regression coefficients are related to the corresponding values $\alpha_0, \alpha_1, \dots$ obtained by Fisher, in this manner:

$$\alpha_i' = \frac{\alpha_i}{\sqrt{S(\xi_i'^2)}}$$

To obtain values for the regression function a , Fisher uses

$$a = \alpha_0 T_0 + \alpha_1 T_1 + \dots$$

In terms of the polynomials ξ_0', ξ_1', \dots and the writers' coefficients $\alpha_0', \alpha_1', \text{etc.}$, this becomes

$$a = \alpha_0' \xi_0' + \alpha_1' \xi_1' + \dots \quad (6)$$

by virtue of the relations previously stated and summarized as follows:

$$\alpha_i' \xi_i' = \frac{\alpha_i}{\sqrt{S(\xi_i'^2)}} T_i \sqrt{S(\xi_i'^2)} = \alpha_i T_i$$

This means that the orthogonal polynomials tabulated by Fisher and Yates (3) are convenient not only for fitting polynomials but also in the expansion of the regression integral and subsequent evaluation of the function a .)

ANALYSIS OF THE CORN DATA

From the rainfall in each of the twenty-four 5-day periods of each season designated by $r_1 \dots r_{24}$, the values of $\rho_0', \rho_1', \dots \rho_5'$ as tabulated in table 1 were calculated by the use of the ξ_i' 's mentioned above, where

$$\begin{aligned} \rho_0' &= S(r\xi_0') \text{ summed over the 24 periods} \\ \rho_1' &= S(r\xi_1') \text{ summed over the 24 periods} \\ &\vdots \\ \rho_5' &= S(r\xi_5') \text{ summed over the 24 periods} \end{aligned}$$

using the tabular values of ξ_i' 's for n equals 24. As ξ_0' is a constant, 1, the value ρ_0' will be the sums of $r_1 \dots r_{24}$ which is the total rainfall for the season of 120 days.

As a curve of the fifth degree was being fitted it was worth while to actually work from the center of the 24 values and to build up a column of sums and differences of paired values around the center. The differences were then used with ξ_1', ξ_3' , and ξ_5' while the sums were used with ξ_2' and ξ_4' (see Fisher and Yates (3)).

The object is now to secure the regression of yield on the six series $\rho_0', \dots \rho_5'$. Before doing this it was advisable to look at the yields over the period to see if any slow changes had taken place. As expected on such continuous plots, the tendency has been for the level of yields to decline gradually. A linear regression was used to remove the slow changes since examination showed that it accounted for a major portion of the variation. It is recognized that in other problems slow changes may be expressed better by a parabola or some polynomial of higher degree.

TABLE 1.—*Corn yields from continuous plot 5 at Wooster, Ohio, 1905–36, seasonal rainfall distribution coefficients, and values of ξ_1' for $n=32$*

Year	Yield per acre	ρ_0'	ρ_1'	ρ_2'	ρ_3'	ρ_4'	ρ_5'	ξ_1'
	<i>Bushels</i>							
1905	45.7	22.47	18.75	-738.4	4,507	765	10,705	31
1906	34.3	17.10	92.30	239.2	902	-98	3,481	29
1907	17.7	13.96	-24.96	-263.5	416	-85	-5,147	27
1908	20.8	13.99	-4.07	262.4	-2,252	-437	-1,258	25
1909	19.4	19.73	-3.89	213.0	2,403	496	9,202	23
1910	12.8	9.55	-77.23	82.0	1,896	-280	-3,802	21
1911	32.0	11.62	6.86	-364.8	743	-318	2,926	19
1912	31.6	20.32	28.82	306.3	-3,824	-331	2,150	17
1913	22.9	12.17	45.03	317.2	2,184	199	7,406	15
1914	22.9	14.69	-6.29	90.8	2,217	368	5,661	13
1915	32.9	21.50	68.68	-844.6	-2,352	6	-3,452	11
1916	19.0	12.83	-38.03	-404.8	1,167	182	-11,634	9
1917	24.2	13.26	-21.70	-258.3	3,550	226	-4,142	7
1918	16.9	11.57	-42.05	151.2	1,061	-454	-2,918	5
1919	33.1	21.36	40.02	420.7	-6,094	-732	13,180	3
1920	22.3	19.24	52.40	-443.2	4,926	496	-3,142	1
1921	20.1	12.44	-33.26	-115.2	-1,540	153	-6,448	-1
1922	9.6	14.49	-72.85	-412.0	1,044	-192	16,060	-3
1923	20.3	12.82	3.74	603.8	-684	-464	12,692	-5
1924	14.1	17.38	-49.88	-220.8	628	40	472	-7
1925	27.3	11.12	16.16	-44.0	-3,864	25	3,377	-9
1926	29.8	11.00	12.86	-105.2	1,606	356	4,317	-11
1927	21.1	15.06	-18.74	-22.4	-2,142	-523	2,238	-13
1928	12.1	14.55	43.27	-499.1	528	-196	-7,442	-15
1929	9.9	15.76	-55.00	-772.0	-1,852	700	4,762	-17
1930	15.0	6.91	10.07	-352.0	-734	102	1,830	-19
1931	34.1	15.25	10.49	364.5	3,353	-117	11,998	-21
1932	23.7	10.52	4.08	-171.0	-1,852	421	6,670	-23
1933	6.9	12.01	-40.71	471.7	-3,335	-288	-5,066	-25
1934	25.6	11.77	103.85	46.4	841	584	2,968	-27
1935	29.0	27.89	95.45	-157.9	-14,772	-72	-20,027	-29
1936	21.1	12.10	30.02	-135.3	-2,733	-375	5,463	-31
Sum	725.3	476.43	184.59	-2,746.3	-14,058	790	40,777	0
Mean	22.67	14.89	5.77	-85.8	-439.3	24.7	1,274.3	---

Although it was not expected that there would be any marked sign of change in the six series of values $\rho_0' \dots \rho_5'$ they were examined. No changes were evident. As a linear regression is being fitted to the yields, it is also fitted to the series $\rho_0' \dots \rho_5'$ in order not to disturb the tests of significance of the regression coefficients.

As the individual deviations of yield from the regression line are not needed elsewhere, the sums of squares and of products with $\rho_0' \dots \rho_5'$ were obtained without calculating the six series of individual deviations from trend. The ξ_1' values of the first order for n equals 32 were employed in the following way:

$$S(y - E_{y|\xi_1'})^2 = S(y^2) - (Sy)^2/n - (S\xi_1'y)^2/S(\xi_1'^2)$$

and

$$S(y - E_{y|\xi_1'})(\rho_0' - E_{\rho_0'|\xi_1'}) = S(y\rho_0') - (Sy)(S\rho_0')/n - S\xi_1'y S\xi_1'\rho_0'/S(\xi_1'^2)$$

* $E_{y|\xi_1'}$ is the estimated value from the regression of y on ξ_1' .

because the sum of the squared deviations from linear regression

$$\begin{aligned}
 &= S(y^2) - (Sy)^2/n - b_{y\xi_1'}^2 S(\xi_1'^2) \\
 &= S(y^2) - (Sy)^2/n - \frac{(S\xi_1'y)^2}{[S(\xi_1'^2)]^2} S(\xi_1'^2) \\
 &= S(y^2) - (Sy)^2/n - (S\xi_1'y)^2/S(\xi_1'^2)
 \end{aligned}$$

The computation of the sums of squares and products of deviations from linear trend line by the above expression from values in table 2 are illustrated below. The value 10,912 for $S(\xi_1'^2)$ is obtained directly from the foot of the tables of ξ 's for n equals 32 in the statistical tables compiled by Fisher and Yates (3).

TABLE 2.—Sums of squares and of products of yield ρ_i' and ξ_1' from table 1

Variable	Y	ρ_0'	ρ_1'	ρ_2'	ρ_3'	ρ_4'	ρ_5'	ξ_1'
y	18,774.35	11,350.83	11,774.50	-63,574.14	-346,358.4	35,069.5	1,360,207.8	1,423.9
ρ_0'		7,703.88	5,460.0	-48,195	-391,631	12,781	494,234	459.03
ρ_1'			68,364	12,876	-1,498,948	60,293	-334,207	-2,775.33
ρ_2'				4,479,341	-4,286,861	-2,342,311	15,563,474	2,304.5
ρ_3'					425,477,142	17,538,629	269,832,155	853,872
ρ_4'						4,553,478	895,211	12,742
ρ_5'							1,952,539,937	227,561
ξ_1'								10,912

For sums of squares:

$$S(y - E_{y\xi_1'})^2 = 18,774.35 - \frac{(725.3)^2}{32} - \frac{(1,423.9)^2}{10,912} = 2,149.17$$

and for sums of products:

$$\begin{aligned}
 S(y - E_{y\xi_1'})(\rho_0' - E_{\rho_0'\xi_1'}) &= 11,350.834 - \frac{(725.3)(476.43)}{32} \\
 &\quad - \frac{(1,423.9)(459.03)}{10,912} = 492.352
 \end{aligned}$$

These and the remaining values of the sums of cross products of yield with the rainfall distribution coefficients ρ_0' . . . ρ_5' with linear trend removed from each are:

$$\begin{aligned}
 S(y\rho_0') &= 492.35 & S(y\rho_3') &= -139,146.2 \\
 S(y\rho_1') &= 7,952.80 & S(y\rho_4') &= 15,501.0 \\
 S(y\rho_2') &= -1,628.25 & S(y\rho_5') &= 406,227.3
 \end{aligned}$$

Here and in succeeding pages relating to corn the notations y and ρ_i' refer to values with trend eliminated. The values of the sums of squares and products of deviations from linear trends fitted to the six series of rainfall distribution coefficients are shown in table 3.

TABLE 3.—Wooster, Ohio, rainfall distribution coefficients: Sums of squares and products corrected for contributions of linear trend

DIRECT						
Variable	ρ_0'	ρ_1'	ρ_2'	ρ_3'	ρ_4'	ρ_5'
ρ_0'	591.28	2,828.4	-7,404	-221,248	483	-122,445
ρ_1'	2,828.4	66,594	29,304	-1,200,693	58,977	-511,549
ρ_2'	-7,404	29,304	4,243,161	-5,673,674	-2,277,203	19,014,974
ρ_3'	-221,248	-1,200,693	-5,673,674	352,485,177	16,888,615	269,939,185
ρ_4'	483	58,977	-2,277,203	16,888,615	4,519,096	-377,195
ρ_5'	-122,445	-511,549	19,014,974	269,939,185	-377,195	1,895,832,968

TABLE 3.—*Wooster, Ohio, rainfall distribution coefficients: Sums of squares and products corrected for contributions of linear trend—Continued*

IN CODE						
Variable	$\rho_0'/10^1$	$\rho_1'/10^2$	$\rho_2'/10^3$	$\rho_3'/10^4$	$\rho_4'/10^5$	$\rho_5'/10^6$
$\rho_0'/10^1$	5.9128	2.8284	-0.7404	-2.2125	0.0483	-1.2244
$\rho_1'/10^2$	2.8284	6.6594	.2930	-1.2007	.5898	-.5115
$\rho_2'/10^3$	-.7404	.2930	4.2432	-.5674	-2.2772	1.9015
$\rho_3'/10^4$	-2.2125	-1.2007	-.5674	3.5248	1.6889	2.6994
$\rho_4'/10^5$.0483	.5898	-2.2772	1.6889	4.5191	-.0377
$\rho_5'/10^6$	-1.2244	-.5115	1.9015	2.6994	-.0377	18.9583

The partial regression coefficients α_0' , α_1' , . . . α_5' of yield on rainfall distribution coefficients ρ_0' . . . ρ_5' may be obtained from the solution of the six simultaneous equations:

$$\begin{aligned} \alpha_0' S(\rho_0'^2) + \alpha_1' S(\rho_0' \rho_1') + \alpha_2' S(\rho_0' \rho_2') + \dots + \alpha_5' S(\rho_0' \rho_5') &= S(y \rho_0') \\ \vdots \\ \alpha_0' S(\rho_0' \rho_5') + \alpha_1' S(\rho_1' \rho_5') + \alpha_2' S(\rho_2' \rho_5') + \dots + \alpha_5' S(\rho_5'^2) &= S(y \rho_5') \quad (7) \end{aligned}$$

As the rainfall distribution coefficients may eventually be correlated with yield data from more than one plot, it was advantageous to calculate at this stage the values of the multipliers C_{rs} . (See 2, sec. 29, for a discussion of C values.)

The solution for these C values appears in table 4 from the six sets of six simultaneous equations in the following manner:

$$\begin{aligned} A_0. \quad & C_{r0} S(\rho_0'^2) + C_{r1} S(\rho_0' \rho_1') + C_{r2} S(\rho_0' \rho_2') + C_{r3} S(\rho_0' \rho_3') \\ & + C_{r4} S(\rho_0' \rho_4') + C_{r5} S(\rho_0' \rho_5') = 0, 0, 0, 0, 1 \\ A_1. \quad & C_{r0} S(\rho_1' \rho_0') + C_{r1} S(\rho_1'^2) + C_{r2} S(\rho_1' \rho_2') + C_{r3} S(\rho_1' \rho_3') \\ & + C_{r4} S(\rho_1' \rho_4') + C_{r5} S(\rho_1' \rho_5') = 0, 0, 0, 0, 1, 0 \\ A_2. \quad & C_{r0} S(\rho_2' \rho_0') + C_{r1} S(\rho_2' \rho_1') + C_{r2} S(\rho_2'^2) + C_{r3} S(\rho_2' \rho_3') \\ & + C_{r4} S(\rho_2' \rho_4') + C_{r5} S(\rho_2' \rho_5') = 0, 0, 0, 1, 0, 0 \\ A_3. \quad & C_{r0} S(\rho_3' \rho_0') + C_{r1} S(\rho_3' \rho_1') + C_{r2} S(\rho_3' \rho_2') + C_{r3} S(\rho_3'^2) \\ & + C_{r4} S(\rho_3' \rho_4') + C_{r5} S(\rho_3' \rho_5') = 0, 0, 1, 0, 0, 0 \\ A_4. \quad & C_{r0} S(\rho_4' \rho_0') + C_{r1} S(\rho_4' \rho_1') + C_{r2} S(\rho_4' \rho_2') + C_{r3} S(\rho_4' \rho_3') \\ & + C_{r4} S(\rho_4'^2) + C_{r5} S(\rho_4' \rho_5') = 0, 1, 0, 0, 0, 0 \\ A_5. \quad & C_{r0} S(\rho_5' \rho_0') + C_{r1} S(\rho_5' \rho_1') + C_{r2} S(\rho_5' \rho_2') + C_{r3} S(\rho_5' \rho_3') \\ & + C_{r4} S(\rho_5' \rho_4') + C_{r5} S(\rho_5'^2) = 1, 0, 0, 0, 0, 0 \end{aligned}$$

In the first set of equations to be solved, equation A_5 is set equal to 1 and the others are set equal to 0. In succeeding sets a similar procedure is followed with a different equation being set equal to 1. The subscript r for the C values in any set of the equations follows that of the equation that is set equal to 1. The scheme of the solution followed in table 4 is that set out by Waugh (8).

The C values can be obtained in terms of the reduced equations in table 4. For the first set of six simultaneous equations these are obtained as follows:

$$\begin{aligned} C_{55} &= 1/a_{55} = 0.06557213 \\ C_{45} &= a_{45}'' C_{55} = 0.00835061 \\ C_{05} &= a_{05}'' C_{55} + a_{04}'' C_{44} + a_{03}'' C_{33} + a_{02}'' C_{22} + a_{01}'' C_{11} = -0.01840803 \end{aligned}$$

TABLE 4.—Reduction of the simultaneous equations $A_0 \dots A_5$

Equation	Line	Reciprocal	0	1	2	3	4	5	Check
A_0	$\{a_0, a_0', a_0'', a_0'''\}$	$\{-0.16912461\}$	$\{5.9128, 5.9128, -1.000000\}$	$\{2.5254, 2.5254, -1.75332, 6.6391, 5.306429, -1.990000\}$	$\{-0.7404, -7.404, -125220, 2630, 647172, -121990, 4.2432, 4.071558, -1.000000\}$	$\{-2.2123, -2.2123, 371138, -1.2007, 122146, 026823, -5674, -827050, 203138, 3.5248, 2.525078, -1.000000\}$	$\{0.0483, 0.0483, -0.06169, -3898, -569206, -106794, -2.2772, -2.340266, -374784, 1.6889, 1.246778, -493728, 4.5191, -1.000000\}$	$\{-1.2244, -1.2244, -207376, -5115, -074194, -013982, 1.9015, 1.739132, -437142, 2.6991, 2.596518, -1.0296202, -0377, -318048, -127350, 18.9583, 15.250381, -1.000000\}$	$\{4.6122, 4.6122, -780037, 8.6584, 6.452145, -1.215911, 2.8527, 2.043336, -649220, 3.9225, 6.368371, -2.522019, 4.5312, 2.179386, -872651, 21.7856, 15.250382, -1.000000\}$
A_1	$\{a_1, a_1', a_1'', a_1'''\}$	$\{-1.18845065\}$							
A_2	$\{a_2, a_2', a_2'', a_2'''\}$	$\{-24560022\}$							
A_3	$\{a_3, a_3', a_3'', a_3'''\}$	$\{-39602737\}$							
A_4	$\{a_4, a_4', a_4'', a_4'''\}$	$\{-40041130\}$							
A_5	$\{a_5, a_5', a_5'', a_5'''\}$	$\{-06557213\}$							

The values C_{r4} , C_{r3} , etc., are calculated by a similar process and the final figure to be calculated is:

$$C_{00} = 1/a_{00}' + a_{05}''C_{05} + a_{04}''C_{04} + a_{03}''C_{03} + a_{02}''C_{02} + a_{01}''C_{01} = 0.28956313$$

The $1/a_{rr}'$ are to be obtained in the solution in table 4 and entered (with sign changed) to the left of the a_{rr} values.

For convenience, a coding process was used on the sums of squares and products in table 3 as four or five significant figures were thought adequate. The coding was performed by divisions of powers of 10 by setting the decimal to the left the appropriate number of places. By inspection of the sums of squares, a divisor of a power of 10 best suited for each coefficient was selected. Remembering that exponents are added when multiplying numbers, the power of 10 is doubled in case of a sum of squares, and the exponents are added in case of a sum of products. Thus, for ρ_2' the divisor is 10^3 which becomes 10^6 on squaring and the sum of squares 4,243,162.10 is coded to 4.2432; and for the product of $\rho_1'\rho_2'$ the divisors are 10^2 and 10^3 which on multiplying become 10^5 and the sum of products of 29,303.646 is coded to 0.2930. The lower part of table 3 contains the coded values. Upon completion of the solution in table 5 it is

TABLE 5.—Values of C_{rs} , in code

$r \backslash s$	$C_{r0} \cdot 10^1$	$C_{r1} \cdot 10^2$	$C_{r2} \cdot 10^3$	$C_{r3} \cdot 10^4$	$C_{r4} \cdot 10^5$	$C_{r5} \cdot 10^6$
$C_{00} \cdot 10^4$	0.289563	-0.092118	0.078552	0.187531	-0.021728	-0.018408
$C_{10} \cdot 10^2$	-.092118	.204791	-.064093	.035030	-.071125	.000875
$C_{20} \cdot 10^3$.078552	-.064093	.373820	.027825	.185182	-.037743
$C_{30} \cdot 10^4$.187531	.035030	.027825	.571720	-.206818	-.071550
$C_{40} \cdot 10^5$	-.021728	-.071125	.185182	-.206818	.401475	.008351
$C_{50} \cdot 10^6$	-.018408	.000875	-.037743	-.071550	.008351	.065572

necessary to decode. Owing to the nature of the C values, the decoding is done by dividing again by the same powers of 10 rather than by multiplying as might be expected. The decoded C values appear in table 6.

TABLE 6.—Values of C_{rs} , decoded

$r \backslash s$	C_{r0}	C_{r1}	C_{r2}	C_{r3}	C_{r4}	C_{r5}
C_{00}	-0.00289563	-0.092118	0.078552	0.0187531	-0.0021728	-0.0018408
C_{10}	-.092118	.0204791	-.064093	.035030	-.071125	.000875
C_{20}	.078552	-.064093	.0373820	.027825	.0185182	-.037743
C_{30}	.0187531	.035030	.027825	.0571720	-.0206818	-.071550
C_{40}	-.0021728	-.071125	.0185182	-.0206818	.0401475	.008351
C_{50}	-.0018408	.000875	-.037743	-.071550	.008351	.065572

The partial regression coefficient α_s' of any dependent variate y on ρ_s' may then be rapidly obtained from the equation:

$$\alpha_s' = C_{0s}S(\rho_0'y) + C_{1s}S(\rho_1'y) + C_{2s}S(\rho_2'y) + C_{3s}S(\rho_3'y) + C_{4s}S(\rho_4'y) + C_{5s}S(\rho_5'y) \quad (8)$$

The six regression coefficients are:

$$\begin{aligned}\alpha_0' &= 0.310872 \\ \alpha_1' &= 0.103012 \\ \alpha_2' &= -0.000888\end{aligned}$$

$$\begin{aligned}\alpha_3' &= -0.000211 \\ \alpha_4' &= 0.002412 \\ \alpha_5' &= 0.00030138\end{aligned}$$

These regression coefficients enable us in the present case to estimate the average effect on the crop of an extra inch of rainfall at any time within the 120-day period. Figure 1 shows the course of the effect

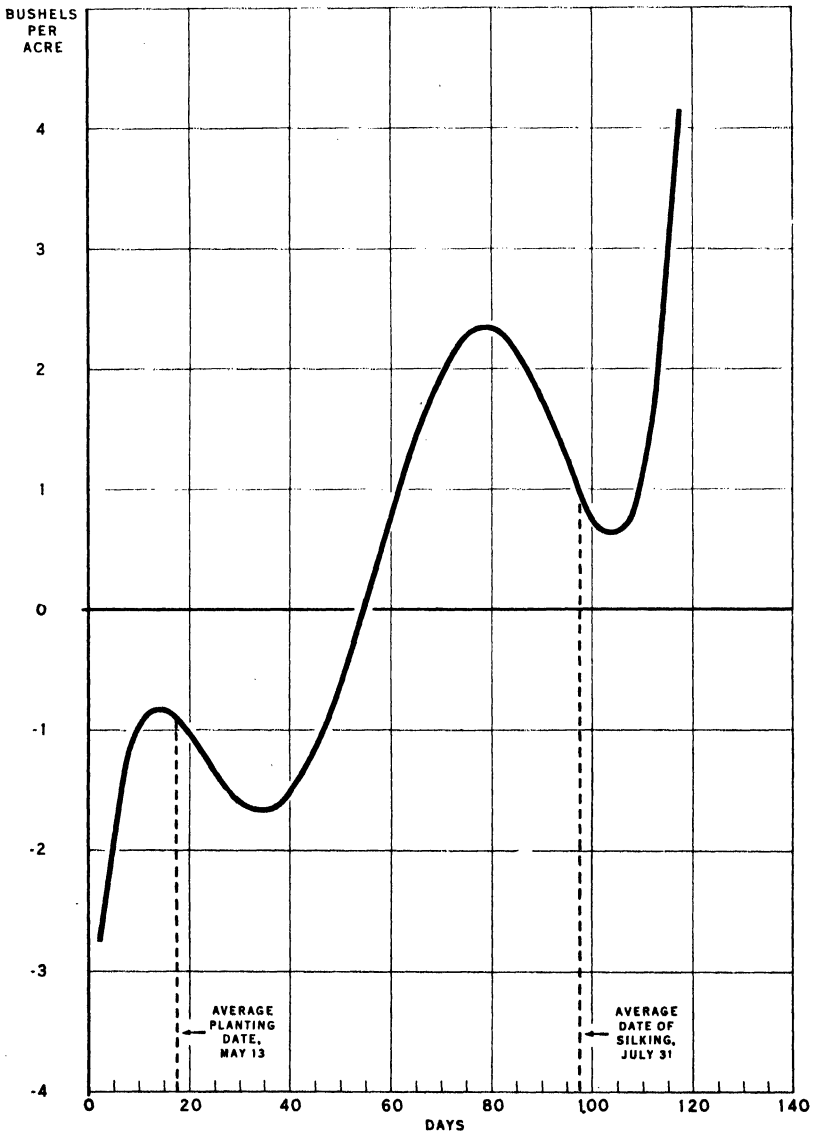


FIGURE 1.—Average effect in bushels per acre of each additional inch of rainfall on the yield of corn on continuously cropped plot No. 5 at Wooster, Ohio.

because the sum of the squared deviations from linear regression

$$\begin{aligned} &= S(y^2) - (Sy)^2/n - b_{y\xi_1'}^2 S(\xi_1'^2) \\ &= S(y^2) - (Sy)^2/n - \frac{(S\xi_1'y)^2}{[S(\xi_1'^2)]^2} S(\xi_1'^2) \\ &= S(y^2) - (Sy)^2/n - (S\xi_1'y)^2/S(\xi_1'^2) \end{aligned}$$

The computation of the sums of squares and products of deviations from linear trend line by the above expression from values in table 2 are illustrated below. The value 10,912 for $S(\xi_1'^2)$ is obtained directly from the foot of the tables of ξ 's for n equals 32 in the statistical tables compiled by Fisher and Yates (3).

TABLE 2.—Sums of squares and of products of yield ρ_i' and ξ_1' from table 1

Variable	Y	ρ_0'	ρ_1'	ρ_2'	ρ_3'	ρ_4'	ρ_5'	ξ_1'
y	18,774.35	11,350.83	11,774.50	-63,574.14	-346,358.4	35,069.5	1,360,207.8	1,423.9
ρ_0'		7,703.88	5,460.0	-48,195	-391,631	12,781	494,234	459.03
ρ_1'			68,364	12,876	-1,498,948	60,293	-334,207	-2,775.33
ρ_2'				4,479,341	-4,286,861	-2,342,311	15,563,474	2,304.5
ρ_3'					425,477,142	17,538,629	269,832,155	853,872
ρ_4'						4,553,478	895,211	12,742
ρ_5'							1,952,539,937	227,561
ξ_1'								10,912

For sums of squares:

$$S(y - E_{y\xi_1'})^2 = 18,774.35 - \frac{(725.3)^2}{32} - \frac{(1,423.9)^2}{10,912} = 2,149.17$$

and for sums of products:

$$\begin{aligned} S(y - E_{y\xi_1'})(\rho_0' - E_{\rho_0'\xi_1'}) &= 11,350.834 - \frac{(725.3)(476.43)}{32} \\ &\quad - \frac{(1,423.9)(459.03)}{10,912} = 492.352 \end{aligned}$$

These and the remaining values of the sums of cross products of yield with the rainfall distribution coefficients ρ_0' . . . ρ_5' with linear trend removed from each are:

$$\begin{aligned} S(y\rho_0') &= 492.35 & S(y\rho_3') &= -139,146.2 \\ S(y\rho_1') &= 7,952.80 & S(y\rho_4') &= 15,501.0 \\ S(y\rho_2') &= -1,628.25 & S(y\rho_5') &= 406,227.3 \end{aligned}$$

Here and in succeeding pages relating to corn the notations y and ρ_i' refer to values with trend eliminated. The values of the sums of squares and products of deviations from linear trends fitted to the six series of rainfall distribution coefficients are shown in table 3.

TABLE 3.—Wooster, Ohio, rainfall distribution coefficients: Sums of squares and products corrected for contributions of linear trend

DIRECT						
Variable	ρ_0'	ρ_1'	ρ_2'	ρ_3'	ρ_4'	ρ_5'
ρ_0'	591.28	2,828.4	-7,404	-221,248	483	-122,445
ρ_1'	2,828.4	66,594	29,304	-1,200,683	58,977	-511,549
ρ_2'	-7,404	29,304	4,243,161	-5,673,674	-2,277,203	19,014,974
ρ_3'	-221,248	-1,200,683	-5,673,674	352,485,177	16,888,615	269,939,185
ρ_4'	483	58,977	-2,277,203	16,888,615	4,519,096	-377,195
ρ_5'	-122,445	-511,549	19,014,974	269,939,185	-377,195	1,895,832,968

TABLE 3.—*Wooster, Ohio, rainfall distribution coefficients: Sums of squares and products corrected for contributions of linear trend—Continued*

IN CODE						
Variable	$\rho_0'/10^1$	$\rho_1'/10^2$	$\rho_2'/10^3$	$\rho_3'/10^4$	$\rho_4'/10^5$	$\rho_5'/10^6$
$\rho_0'/10^1$	5.9128	2.8284	-0.7404	-2.2125	0.0483	-1.2244
$\rho_1'/10^2$	2.8284	6.6594	.2930	-1.2007	.5898	-.5115
$\rho_2'/10^3$	-.7404	.2930	4.2432	-.5674	-2.2772	1.9015
$\rho_3'/10^4$	-2.2125	-1.2007	-.5674	3.5248	1.6889	2.6994
$\rho_4'/10^5$.0483	.5898	-2.2772	1.6889	4.5191	-.0377
$\rho_5'/10^6$	-1.2244	-.5115	1.9015	2.6994	-.0377	18.9583

The partial regression coefficients α_0' , α_1' , . . . α_5' of yield on rainfall distribution coefficients ρ_0' , . . . ρ_5' may be obtained from the solution of the six simultaneous equations:

$$\begin{aligned} \alpha_0' S(\rho_0'^2) + \alpha_1' S(\rho_0' \rho_1') + \alpha_2' S(\rho_0' \rho_2') + \dots + \alpha_5' S(\rho_0' \rho_5') &= S(y \rho_0') \\ \vdots \\ \alpha_0' S(\rho_0' \rho_5') + \alpha_1' S(\rho_1' \rho_5') + \alpha_2' S(\rho_2' \rho_5') + \dots + \alpha_5' S(\rho_5'^2) &= S(y \rho_5') \quad (7) \end{aligned}$$

As the rainfall distribution coefficients may eventually be correlated with yield data from more than one plot, it was advantageous to calculate at this stage the values of the multipliers C_{rs} . (See 2, sec. 29, for a discussion of C values.)

The solution for these C values appears in table 4 from the six sets of six simultaneous equations in the following manner:

$$\begin{aligned} A_0. \quad & C_{r0} S(\rho_0'^2) + C_{r1} S(\rho_0' \rho_1') + C_{r2} S(\rho_0' \rho_2') + C_{r3} S(\rho_0' \rho_3') \\ & + C_{r4} S(\rho_0' \rho_4') + C_{r5} S(\rho_0' \rho_5') = 0, 0, 0, 0, 1 \\ A_1. \quad & C_{r0} S(\rho_1' \rho_0') + C_{r1} S(\rho_1'^2) + C_{r2} S(\rho_1' \rho_2') + C_{r3} S(\rho_1' \rho_3') \\ & + C_{r4} S(\rho_1' \rho_4') + C_{r5} S(\rho_1' \rho_5') = 0, 0, 0, 0, 1, 0 \\ A_2. \quad & C_{r0} S(\rho_2' \rho_0') + C_{r1} S(\rho_2' \rho_1') + C_{r2} S(\rho_2'^2) + C_{r3} S(\rho_2' \rho_3') \\ & + C_{r4} S(\rho_2' \rho_4') + C_{r5} S(\rho_2' \rho_5') = 0, 0, 0, 1, 0, 0 \\ A_3. \quad & C_{r0} S(\rho_3' \rho_0') + C_{r1} S(\rho_3' \rho_1') + C_{r2} S(\rho_3' \rho_2') + C_{r3} S(\rho_3'^2) \\ & + C_{r4} S(\rho_3' \rho_4') + C_{r5} S(\rho_3' \rho_5') = 0, 0, 1, 0, 0, 0 \\ A_4. \quad & C_{r0} S(\rho_4' \rho_0') + C_{r1} S(\rho_4' \rho_1') + C_{r2} S(\rho_4' \rho_2') + C_{r3} S(\rho_4' \rho_3') \\ & + C_{r4} S(\rho_4'^2) + C_{r5} S(\rho_4' \rho_5') = 0, 1, 0, 0, 0, 0 \\ A_5. \quad & C_{r0} S(\rho_5' \rho_0') + C_{r1} S(\rho_5' \rho_1') + C_{r2} S(\rho_5' \rho_2') + C_{r3} S(\rho_5' \rho_3') \\ & + C_{r4} S(\rho_5' \rho_4') + C_{r5} S(\rho_5'^2) = 1, 0, 0, 0, 0, 0 \end{aligned}$$

In the first set of equations to be solved, equation A_5 is set equal to 1 and the others are set equal to 0. In succeeding sets a similar procedure is followed with a different equation being set equal to 1. The subscript r for the C values in any set of the equations follows that of the equation that is set equal to 1. The scheme of the solution followed in table 4 is that set out by Waugh (8).

The C values can be obtained in terms of the reduced equations in table 4. For the first set of six simultaneous equations these are obtained as follows:

$$\begin{aligned} C_{55} &= 1/a_{55} = 0.06557213 \\ C_{45} &= a_{45}'' C_{55} = 0.00835061 \\ C_{05} &= a_{05}'' C_{55} + a_{04}'' C_{44} + a_{03}'' C_{33} + a_{02}'' C_{22} + a_{01}'' C_{11} = -0.01840803 \end{aligned}$$

TABLE 4.—Reduction of the simultaneous equations $A_0 \dots A_5$

Equation	Line	Reciprocal	0	1	2	3	4	5	Check
A_0	$\{a_0, a_0', a_0'', a_0'''\}$	$\{-0.16912461\}$	$\{5.9128, 5.9128, -1.000000\}$	$\{2.5254, 2.5254, -1.75332, 6.6391, 5.306429, -1.990000\}$	$\{-0.7404, -7.404, -125220, 2630, 647172, -121990, 4.2432, 4.071558, -1.000000\}$	$\{-2.2123, -2.2123, 371138, -1.2007, 122146, 026823, -5674, -827050, 203138, 3.5248, 2.525078, -1.000000\}$	$\{0.0483, 0.0483, -0.06169, -3898, -569206, -106794, -2.2772, -2.340266, -374784, 1.6889, 1.246778, -493728, 4.5191, -1.000000\}$	$\{-1.2244, -1.2244, -207376, -5115, -074194, -013982, 1.9015, 1.739132, -437142, 2.6991, 2.596518, -1.0296202, -0377, -318048, -127350, 18.9583, 15.250381, -1.000000\}$	$\{4.6122, 4.6122, -780037, 8.6584, 6.452145, -1.215911, 2.8527, 2.043336, -649220, 3.9225, 6.368371, -2.522019, 4.5312, 2.179386, -872651, 21.7856, 15.250382, -1.000000\}$
A_1	$\{a_1, a_1', a_1'', a_1'''\}$	$\{-1.18845065\}$							
A_2	$\{a_2, a_2', a_2'', a_2'''\}$	$\{-24560022\}$							
A_3	$\{a_3, a_3', a_3'', a_3'''\}$	$\{-39602737\}$							
A_4	$\{a_4, a_4', a_4'', a_4'''\}$	$\{-40041130\}$							
A_5	$\{a_5, a_5', a_5'', a_5'''\}$	$\{-06557213\}$							

The values C_{r4} , C_{r3} , etc., are calculated by a similar process and the final figure to be calculated is:

$$C_{00} = 1/a_{00}' + a_{05}''C_{05} + a_{04}''C_{04} + a_{03}''C_{03} + a_{02}''C_{02} + a_{01}''C_{01} = 0.28956313$$

The $1/a_{rr}'$ are to be obtained in the solution in table 4 and entered (with sign changed) to the left of the a_{rr} values.

For convenience, a coding process was used on the sums of squares and products in table 3 as four or five significant figures were thought adequate. The coding was performed by divisions of powers of 10 by setting the decimal to the left the appropriate number of places. By inspection of the sums of squares, a divisor of a power of 10 best suited for each coefficient was selected. Remembering that exponents are added when multiplying numbers, the power of 10 is doubled in case of a sum of squares, and the exponents are added in case of a sum of products. Thus, for ρ_2' the divisor is 10^3 which becomes 10^6 on squaring and the sum of squares 4,243,162.10 is coded to 4.2432; and for the product of $\rho_1'\rho_2'$ the divisors are 10^2 and 10^3 which on multiplying become 10^5 and the sum of products of 29,303.646 is coded to 0.2930. The lower part of table 3 contains the coded values. Upon completion of the solution in table 5 it is

TABLE 5.—Values of C_{rs} , in code

$r \backslash s$	$C_{r0} \cdot 10^1$	$C_{r1} \cdot 10^2$	$C_{r2} \cdot 10^3$	$C_{r3} \cdot 10^4$	$C_{r4} \cdot 10^5$	$C_{r5} \cdot 10^6$
$C_{00} \cdot 10^4$	0.289563	-0.092118	0.078552	0.187531	-0.021728	-0.018408
$C_{10} \cdot 10^2$	-.092118	.204791	-.064093	.035030	-.071125	.000875
$C_{20} \cdot 10^3$.078552	-.064093	.373820	.027825	.185182	-.037743
$C_{30} \cdot 10^4$.187531	.035030	.027825	.571720	-.206818	-.071550
$C_{40} \cdot 10^5$	-.021728	-.071125	.185182	-.206818	.401475	.008351
$C_{50} \cdot 10^6$	-.018408	.000875	-.037743	-.071550	.008351	.065572

necessary to decode. Owing to the nature of the C values, the decoding is done by dividing again by the same powers of 10 rather than by multiplying as might be expected. The decoded C values appear in table 6.

TABLE 6.—Values of C_{rs} , decoded

$r \backslash s$	C_{r0}	C_{r1}	C_{r2}	C_{r3}	C_{r4}	C_{r5}
C_{00}	-0.00289563	-0.092118	0.078552	0.0187531	-0.0021728	-0.0018408
C_{10}	-.092118	.0204791	-.064093	.035030	-.071125	.000875
C_{20}	.078552	-.064093	.0373820	.027825	.0185182	-.037743
C_{30}	.0187531	.035030	.027825	.0571720	-.0206818	-.071550
C_{40}	-.0021728	-.071125	.0185182	-.0206818	.0401475	.008351
C_{50}	-.0018408	.000875	-.037743	-.071550	.008351	.065572

The partial regression coefficient α_s' of any dependent variate y on ρ_s' may then be rapidly obtained from the equation:

$$\alpha_s' = C_{0s}S(\rho_0'y) + C_{1s}S(\rho_1'y) + C_{2s}S(\rho_2'y) + C_{3s}S(\rho_3'y) + C_{4s}S(\rho_4'y) + C_{5s}S(\rho_5'y) \quad (8)$$

The six regression coefficients are:

$$\alpha_0' = 0.310872$$

$$\alpha_1' = 0.103012$$

$$\alpha_2' = -0.000888$$

$$\alpha_3' = -0.000211$$

$$\alpha_4' = 0.002412$$

$$\alpha_5' = 0.00030138$$

These regression coefficients enable us in the present case to estimate the average effect on the crop of an extra inch of rainfall at any time within the 120-day period. Figure 1 shows the course of the effect

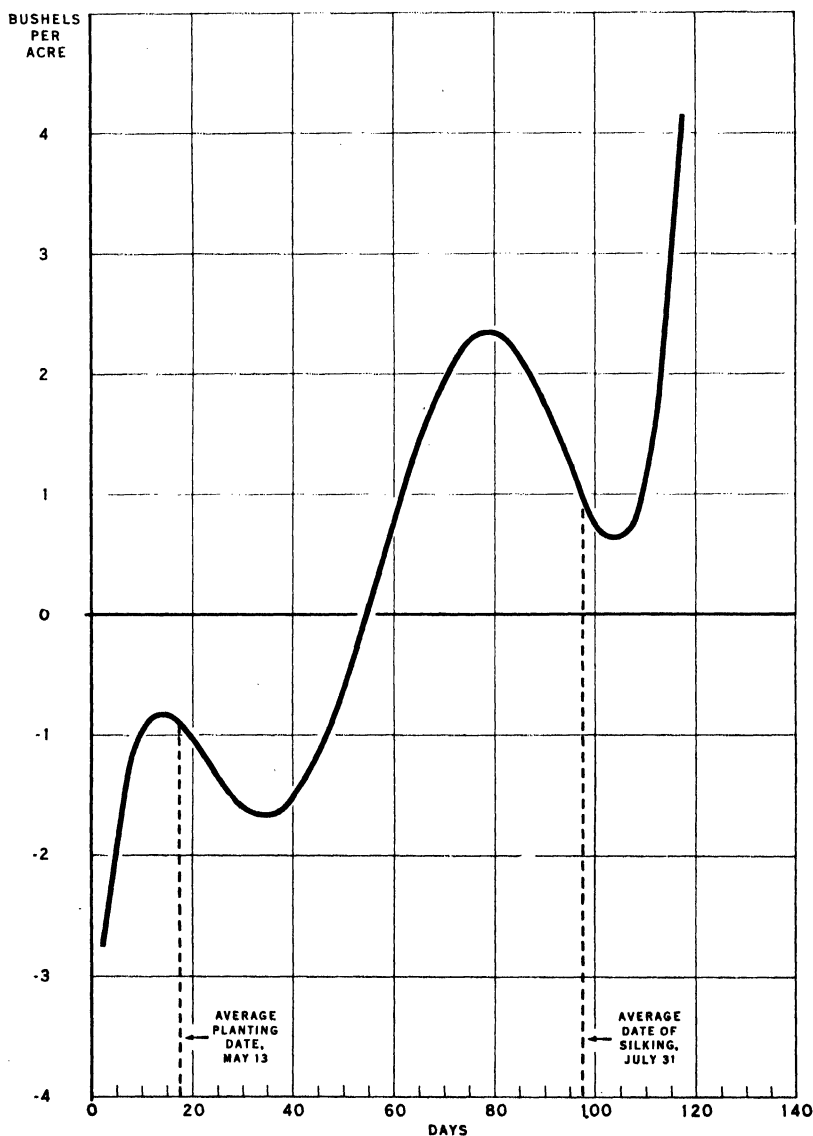


FIGURE 1.—Average effect in bushels per acre of each additional inch of rainfall on the yield of corn on continuously cropped plot No. 5 at Wooster, Ohio.

throughout the 120-day period. This is a continuous curve smoothed from the 24 points found by substituting successively the values of $\xi_0' \dots \xi_5'$ for -23 to $+23$ in equation (6), $a = \alpha_0' \xi_0' + \alpha_1' \xi_1' + \dots$, and using the above calculated values of $\alpha_0' \dots \alpha_5'$.

The significance of the amount of variation in the annual yields accounted for by the regression formula may be investigated by determining the distribution of the total variance of yield, corresponding to 30 degrees of freedom (1 having been used in eliminating trend) between the 6 degrees of freedom of the regression formula and the 24 degrees of freedom appropriate to deviations of yield from the regression formula. From table 7 it is apparent that the regression formula has accounted for 54 percent of the total variance of yield. The ratio of the two mean squares is $F = 4.7(6)$, a highly significant value.

TABLE 7.—*Analysis of variance of yield of corn*

Source	Degrees of freedom	Sums of squares	Mean square
Precipitation regression	6	1,162.93	**193.82
Deviation from regression	24	986.24	41.09
Total	30	2,149.17	
	$R^2 = 0.5411$ $R = .73+$		

**Highly significant.

CONTRIBUTIONS OF THE DIFFERENT TERMS IN THE REGRESSION FORMULA

The $\alpha_0', \alpha_1', \dots, \alpha_5'$ are the coefficients in the representations (by orthogonal polynomials) of the regression function of yield on rainfall. They are also the partial regression coefficients of yield on the 0th, 1st, \dots 5th coefficients of the orthogonal polynomials fitted to each year's rainfall, and such partial regression coefficients represent, in order, the regression of yield on total seasonal rainfall, on the average rate at which the rainfall was increasing or decreasing during the season, on the parabolic term in the rainfall sequence, and so on to the higher terms of the polynomials fitted to the seasonal rainfall. Each of these regression coefficients in the formula may be tested to see if it differs significantly from zero (2, sec. 29). The coefficient α_1' , which represents the regression on the linear component of a polynomial, when tested is highly significant. The t value is 3.55 as compared with 2.797 at the 1-percent level for 24 degrees of freedom. Neither coefficient α_0' , representing the regression on the mean rainfall for the season, nor the coefficients $\alpha_2', \alpha_3', \alpha_4'$ approach significance. The coefficient α_5' approaches the 5-percent level of significance, it having a t value of 1.84 as compared with the tabular value of 2.064. Had yield been studied only in relation to the total rainfall during the season we might have arrived at the misleading conclusion that the total amount affects yield. The present analysis shows that the real effect is from the distribution.

ANALYSIS OF SPRING WHEAT DATA

The analysis of the spring wheat data involves the steps which are necessary in obtaining the regression of yield on the amount and

distribution of rainfall, and also the partial regression of yield on the amount and distribution of evaporation eliminating associated rainfall effect. Whereas six coefficients representing the seasonal amount and distribution of rainfall were used in the corn analysis, only four coefficients are used in the analysis of the spring wheat yields to obtain a regression function of a similar nature.

The decision to use a third-degree polynomial resulted from having first used a fifth-degree polynomial to represent the seasonal amount and distribution of rainfall and finding that the contributions of the fourth and fifth terms were not significant.

The regression of wheat yield on the amount and distribution of rainfall is found first by the method outlined in the previous section.

TABLE 8.—Average spring wheat yields from two continuously cropped plots at Dickinson, N. Dak., and seasonal rainfall distribution coefficients

Year	Yield per acre	ρ_0'	ρ_1'	ρ_2'	ρ_3'	Year	Yield per acre	ρ_0'	ρ_1'	ρ_2'	ρ_3'
	<i>Bushels</i>						<i>Bushels</i>				
1908	21.0	10.48	3.88	-587.5	-192	1922	21.6	11.67	39.59	-599.2	-3,336
1909	26.0	11.14	35.80	-790.6	-61	1923	11.3	12.23	88.79	219.1	-1,714
1910	17.8	7.92	14.10	-8.2	116	1924	16.6	8.10	45.92	-173.7	-1,324
1911	3.6	4.99	2.57	-357.5	-505	1925	5.8	7.75	18.23	-436.1	-469
1913	17.0	5.43	16.55	-195.0	-996	1926	0	6.44	6.62	-385.7	-1,104
1914	9.7	16.79	144.57	338.5	5,945	1927	12.4	11.51	-29.61	-248.2	1,447
1915	27.2	13.42	75.48	-479.9	242	1928	13.8	9.28	49.86	-218.4	-3,900
1916	18.8	11.01	12.03	-282.0	-2,068	1929	9.2	7.51	4.71	-776.2	-787
1917	4.1	4.80	4.02	-205.7	-2,040	1930	6.2	7.48	-13.66	-383.2	-1,651
1918	5.4	7.12	1.56	75.9	1,630	1931	1.1	6.41	45.77	-355.3	-3,742
1919	.6	4.74	-38.12	109.2	1,348	1932	14.7	9.76	4.86	-751.4	-1,480
1920	14.2	9.36	51.50	-271.4	-3,197	1933	1.3	7.27	18.23	-194.6	-971
1921	3.2	7.50	25.70	-270.7	-773	1934	.6	5.51	12.85	-296.1	-3,553

Table 8 includes the annual yield values and the annual rainfall distribution coefficients. It is evident that there is a slight downward trend in yields of these continuously cropped plots. In order to eliminate trend, a linear regression line was fitted in all cases; but because of the missing year, 1912, the ξ' values could not be used as in corn, and individual deviations had to be calculated. In table 9 are

TABLE 9.—Yield and rainfall distribution coefficients from table 8 with linear trend removed

Year	Yield	ρ_0'	ρ_1'	ρ_2'	ρ_3'	Year	Yield	ρ_0'	ρ_1'	ρ_2'	ρ_3'
1908	3.15	0.72	-30.27	-362.7	-809	1922	11.05	3.05	15.33	-306.6	-2,372
1909	8.67	1.46	2.36	-560.9	-565	1923	1.27	3.69	65.24	516.5	-637
1910	.99	-1.67	-18.63	226.3	-275	1924	7.09	-36	23.08	128.5	-135
1911	-12.69	-4.52	-29.46	-118.1	-783	1925	-3.19	-63	-3.91	-129.1	833
1913	1.76	-3.92	-14.06	54.0	-1,048	1926	-8.47	-1.86	-14.81	-73.8	311
1914	-5.02	7.52	114.66	592.4	6,005	1927	4.46	3.29	-50.34	98.5	2,975
1915	13.00	4.23	46.28	-221.2	415	1928	6.38	1.14	29.84	103.1	-2,259
1916	5.12	1.90	-15.56	-18.5	-1,782	1929	2.30	-55	-14.60	-449.8	967
1917	-9.06	-4.23	-23.77	62.7	-1,641	1930	-1.18	-50	-32.27	-52.0	216
1918	-7.24	-1.83	-25.52	349.1	2,142	1931	-4.76	-1.49	27.87	-19.2	-1,762
1919	-11.52	-4.13	-64.50	387.2	1,973	1932	0.36	1.94	-12.34	-410.5	613
1920	2.61	.57	25.83	11.5	-2,459	1933	-3.52	-47	1.74	151.1	1,234
1921	-7.87	-1.21	.74	17.0	78	1934	-3.69	-2.14	-2.93	54.5	-1,235

shown the yields and rainfall coefficients with trend removed, and in future calculations these deviations from linear trend are used instead of the actual values.

The sums of products of deviations from linear trend of yield and rainfall distribution coefficients are:

$$\begin{aligned} S(y\rho_0') &= 310.0348 \\ S(y\rho_1') &= 1,655.3187 \end{aligned}$$

$$\begin{aligned} S(y\rho_2') &= -23,121.527 \\ S(y\rho_3') &= -81,791.340 \end{aligned}$$

The sums and products of deviations of rainfall distribution coefficients appear in table 10. Table 11 displays the rainfall multipliers.

TABLE 10.—Sums of squares and products of deviations of rainfall coefficients

Variable	ρ_0'	ρ_1'	ρ_2'	ρ_3'
ρ_0'	209.7418	1,798.6864	634.926	45,413.89
ρ_1'	1,798.6864	34,787.8892	77,137.194	217,110.13
ρ_2'	634.926	77,137.194	2,005,019.60	5,140,831.1
ρ_3'	45,413.89	217,110.13	5,140,831.1	87,099,870

TABLE 11.—Values of C_r (rainfall) from the sums of squares and products in table 10

r \ s	C_{r0}	C_{r1}	C_{r2}	C_{r3}
C_{00}	0.0116104116	-0.0064333920	0.0038137625	-0.0066551739
C_{10}	-0.0064333920	0.067079232	-0.0033015510	0.0036061272
C_{20}	0.0038137625	-0.0033015510	0.075716799	-0.0055959632
C_{30}	-0.0066551739	0.0036061272	-0.0055959632	0.017236327

These multipliers, or C values, are calculated as outlined in a previous section of this report. The regression of yield on rainfall is then obtained by multiplying the sums of cross products of yield and rainfall distribution coefficients times the appropriate multipliers from table 11, according to equation (8) as follows:

$$310.0348 (0.0116104116) + 1,655.3187 (-0.0064333920) + (-23,121.527) (0.0038137625) + (-81,791.34) (-0.0066551739) = 2.19723569$$

$$310.0348 (-0.0064333920) + 1,655.3187 (0.067079232) + (-23,121.527) (-0.0033015510) + (-81,791.34) (0.0036061272) = -0.04157813$$

$$310.0348 (0.0038137625) + 1,655.3187 (-0.0033015510) + (-23,121.527) (0.075716799) + (-81,791.34) (-0.0055959632) = -0.00657100$$

$$310.0348 (-0.0066551739) + 1,655.3187 (0.0036061272) + (-23,121.527) (-0.0055959632) + (-81,791.34) (0.017236327) = -0.00158232$$

As already explained, these four regression coefficients are used to estimate the average effect on the crop in bushels per acre of an extra inch of rainfall occurring within the 120-day period. Figure 2 shows the course of this function.

The regression of wheat yield on rainfall having been found, we turn our attention to the evaporation records. These are to be treated exactly as were the rainfall records, that is, four distribution coefficients, E_0, E_1, E_2, E_3 , representing the evaporation for the twenty-four 5-day periods for each season were calculated by fitting a third-degree polynomial to the evaporation sequence of each season. The actual values and deviations from linear trend of the four series of distribution coefficients are shown in tables 12 and 13, respectively.

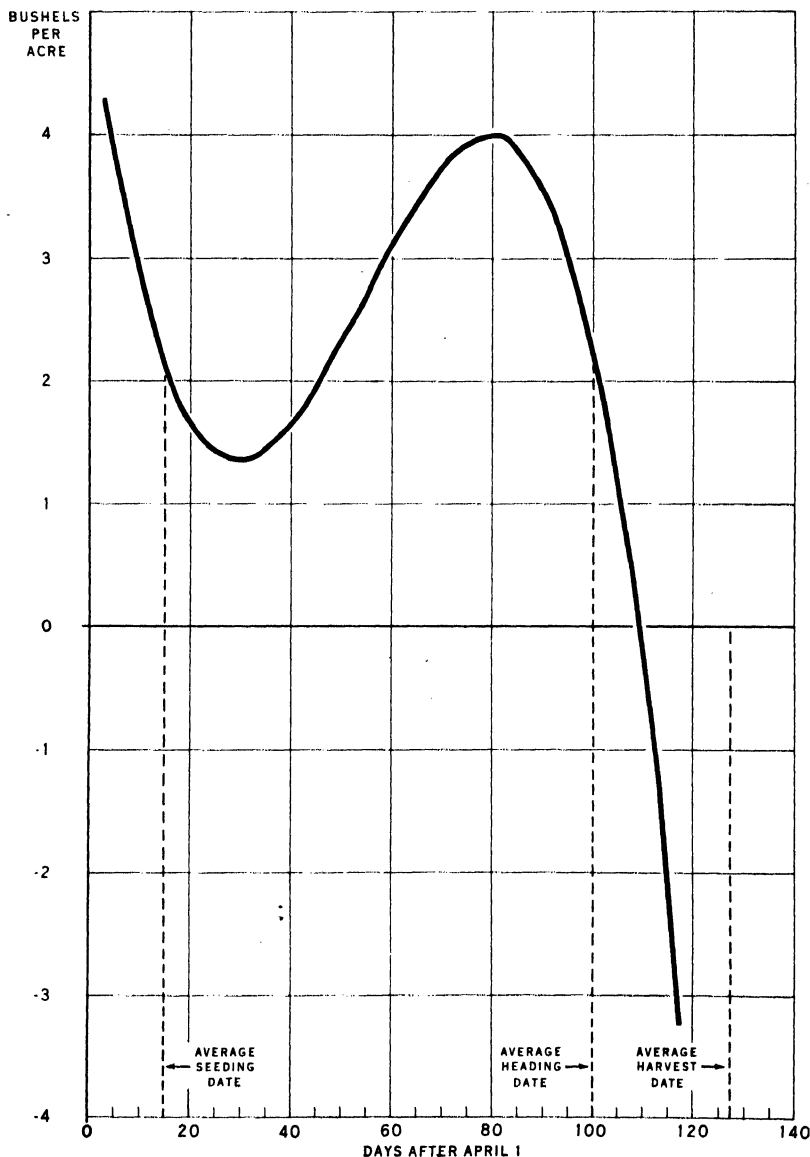


FIGURE 2.—Average effect of an additional inch of rainfall on the yield of spring wheat from continuously cropped plots at Dickinson, N. Dak.

The partial regression of yield on evaporation, eliminating associated rainfall effect, is determined as outlined by Tippett (?) and Hopkins (5). The associated rainfall effect must be removed from the evaporation values, as must also that portion of the variation in yield that is due to rainfall. The residual evaporation values are then correlated with the yield residuals to give the desired relationship

between yield and evaporation with the associated rainfall effect removed. It is well to recognize here that instead of now using this additional factor, handled in much the same manner as rainfall, we might have used a single factor, had we wished, such as the amount of moisture available in the soil at the beginning of the season.

TABLE 12.—*Evaporation distribution coefficients, Dickinson, N. Dak.*

Year	E_0	E_1	E_2	E_3	Year	E_0	E_1	E_2	E_3
1908	20.591	81.947	71.495	-368.841	1922	18.913	40.457	-325.853	814.859
1909	18.084	39.492	-37.416	518.804	1923	20.647	74.721	-352.811	-419.713
1910	25.855	79.529	215.941	-1,880.197	1924	18.220	67.542	102.226	967.524
1911	27.081	71.259	138.837	-1,103.117	1925	23.974	75.048	-52.898	-709.341
1913	21.831	71.171	-15.831	-775.983	1926	25.347	70.593	-55.773	1,884.344
1914	20.746	80.288	39.958	-1,444.484	1927	18.507	69.313	42.237	592.551
1915	18.508	.792	93.772	-1,397.716	1928	19.585	25.653	-217.655	2,984.581
1916	17.718	42.270	-82.212	419.920	1929	22.828	97.140	140.266	1,781.810
1917	23.716	137.874	-59.312	501.918	1930	25.750	126.700	363.322	-1,346.700
1918	21.415	69.221	-323.603	-70.993	1931	28.690	81.638	-75.806	2,824.806
1919	29.259	130.223	-266.649	-1,339.669	1932	22.496	109.736	367.790	573.462
1920	18.584	52.860	-281.080	1,496.780	1933	26.435	139.039	178.379	-1,733.637
1921	24.089	99.495	148.049	-693.185	1934	31.666	57.182	-198.356	2,653.604

TABLE 13.—*Evaporation distribution coefficients from table 12 with linear trend removed*

Year	E_0	E_1	E_2	E_3	Year	E_0	E_1	E_2	E_3
1908	0.14	20.46	110.1	603	1922	-3.91	-36.86	-311.1	575
1909	-2.54	-23.13	-.5	1,404	1923	-2.35	-3.73	-339.7	-746
1910	5.06	15.78	251.1	-1,090	1924	-4.94	-12.05	113.6	555
1911	6.12	6.38	172.3	-391	1925	.64	-5.67	-43.2	-1,268
1913	.53	4.03	14.2	-237	1926	1.85	-11.26	-47.8	1,299
1914	-.72	12.02	68.3	-992	1927	-5.16	-13.67	48.5	-79
1915	-3.13	-68.61	120.4	-1,032	1928	-4.26	-58.46	-213.1	2,226
1916	-4.09	-28.26	-57.2	700	1929	-1.18	11.90	143.1	937
1917	1.74	66.20	-36.0	785	1930	1.57	40.33	394.5	-2,278
1918	-.73	-3.58	-302.0	36	1931	4.34	-5.87	-76.3	1,807
1919	6.94	56.29	-246.8	-1,319	1932	-2.02	21.10	365.6	-531
1920	-3.90	-22.20	-262.9	1,430	1933	1.75	49.27	174.4	-2,924
1921	1.44	23.31	164.5	-846	1934	6.81	-33.72	-204.0	1,376

The sums of products of deviations from linear trend of yield and evaporation distribution coefficients are:

$$S(yE_0) = -486.2268$$

$$S(yE_1) = -3,376.4161$$

$$S(yE_2) = 3,251.988$$

$$S(yE_3) = 41,339.910$$

The sums of squares and products of evaporation distribution coefficients are shown in table 14.

TABLE 14.—*Sums of squares and products of evaporation distribution coefficients*

$r \backslash s$	E_0	E_1	E_2	E_3
E_0	335.8926	1,478.2213	2,155.183	-27,277.65
E_1	1,478.2213	26,603.5280	61,226.360	-530,147.71
E_2	2,155.183	61,226.360	1,041,705.66	-2,974,677.2
E_3	-27,277.65	-530,147.71	-2,974,677.2	40,808.284

To obtain the residual sums of squares and products of the 4 evaporation coefficients with the association of the rainfall coefficients removed, we calculate the regression of the evaporation coefficients on the rainfall coefficients. For the regression of the evaporation coefficients on the rainfall coefficients we obtained the 16 regression coefficients in table 16. These are obtained by multiplying the appro-

TABLE 15.—Sums of cross products of rainfall distribution coefficients and evaporation distribution coefficients

$r \backslash s$	ρ_0'	ρ_1'	ρ_2'	ρ_3'
E_0	-177.2879	-1,155.0458	4,856.3770	6,105.86
E_1	-1,027.3392	-11,907.4509	48,279.3420	448,731.49
E_2	-194.602	-33,971.156	-484,275.46	2,402,066.00
E_3	-4,802.38	86,331.47	-2,723,613.40	-30,737,685.00

TABLE 16.—Regression of evaporation distribution coefficients on rainfall distribution coefficients

Variable	E_0	E_1	E_2	E_3
ρ_0'	-1.1707241	-5.4124242	-14.859716	-10.605535
ρ_1'	.02274494	-1.13539302	.31151172	6.7883564
ρ_2'	.000398751	.01157752	-.39636077	-.81034246
ρ_3'	.000596984	.00757593	.06754736	-.31429949

appropriate figures in table 15 of cross products times the appropriate rainfall multipliers from table 11 in the manner indicated in equation (8). For convenience, the regression coefficient of E_s on ρ_r' is designated by β_{rs} . The 16 regression coefficients of table 16 are used in the following equations to obtain the residual sums of squares and products of the 4 evaporation coefficients with the association of the rainfall coefficients removed:

$$S'(E_s^2) = S(E_s^2) - \beta_{0s}S(\rho_0'E_s) - \beta_{1s}S(\rho_1'E_s) - \beta_{2s}S(\rho_2'E_s) - \beta_{3s}S(\rho_3'E_s)$$

$$S'(E_rE_s) = S(E_rE_s) - \beta_{0s}S(\rho_0'E_r) - \beta_{1s}S(\rho_1'E_r) - \beta_{2s}S(\rho_2'E_r) - \beta_{3s}S(\rho_3'E_r)$$

where $S'(E_s^2)$ is a residual sum of squares and $S'(E_rE_s)$ is a residual sum of products. Such residual sums of squares and products are shown in table 17.

TABLE 17.—Residual sums of squares and cross products of evaporation distribution coefficients

$r \backslash s$	E_{r0}	E_{r1}	E_{r2}	E_{r3}
E_{00}	149.0827	259.7954	1,455.047	-15,462.62
E_{01}	259.7954	15,472.4218	42,982.43	-280,052.25
E_{02}	1,455.047	42,982.43	719,215.99	-2,383,593.6
E_{03}	-15,462.62	-280,052.25	-2,383,593.6	28,393,405

The partial regression coefficients of yield residuals on evaporation residuals could be found by using this table directly; however, the

table of multipliers or C values are calculated as before and are shown in table 18.

TABLE 18.—Values of C_{rs} (evaporation) from the sums of squares and products in table 16

$r \backslash s$	C_{r0}	C_{r1}	C_{r2}	C_{r3}
C_{00}	0.0071553109	-0.06056141	0.0107969	0.033084039
C_{10}	-0.06056141	0.083986663	-0.031320788	0.053246914
C_{20}	0.0107969	-0.031320788	0.020438050	0.014074128
C_{30}	0.033084039	0.053246914	0.014074128	0.0754088115

The yield residuals are obtained by adjusting the sums of products of yield and evaporation by the product of the regression of evaporation on rainfall and the sums of products of yield on rainfall. These calculations are as follows:

$$\begin{aligned}
 & -486.2268 - 310.0348(-1.1707241) - 1,655.3187(0.02274494) - (-23,121.527) \\
 & (0.00038751) - (-81,791.34)(0.00059684) = -103.1356 \\
 & -3,376.4161 - 310.0348(-5.4124242) - 1,655.3187(-0.13539392) - (-23,121.527) \\
 & (0.01157752) - (-81,791.34)(0.00757593) = -586.9207 \\
 & 3,251.988 - 310.0348(-14.859716) - 1,655.3187(0.31151172) - (-23,121.527) \\
 & (-0.39636077) - (-81,791.34)(0.05754736) = 2,885.7754 \\
 & 41,339.91 - 310.0348(-10.605535) - 1,655.3187(6.7883564) - (-23,121.527) \\
 & (-0.81034246) - (-81,791.34)(-0.31429949) = -11,052.24
 \end{aligned}$$

The partial regression coefficients of the yield residuals on the evaporation residuals are then obtained by the same method as outlined previously and are as follows:

$$\begin{aligned}
 & -103.1356(0.0071553109) + (-586.9207)(-0.06056141) + 2,885.7754 \\
 & (0.0107969) + (-11,052.24)(0.033084039) = -0.73867623 \\
 & -103.1356(-0.06056141) + (-586.9207)(0.083986663) + 2,885.7754 \\
 & (-0.031320788) + (-11,052.24)(0.053246914) = -0.05797093 \\
 & -103.1356(0.0107969) + (-586.9207)(-0.031320788) + 2,885.7754 \\
 & (0.020438050) + (-11,052.24)(0.014074128) = 0.00616959 \\
 & -103.1356(0.033084039) + (-586.9207)(0.053246914) + 2,885.7754 \\
 & (0.014074128) + (-11,052.24)(0.0754088115) = -0.00084537
 \end{aligned}$$

These partial regression coefficients are used in equation (6) and the course of the regression function for the effect of evaporation throughout the season is obtained as it appears in figure 3.

TABLE 19.—Analysis of variance of yield of spring wheat

Source	Degrees of freedom	Sums of squares	Mean square	F
Rainfall regression formula	4	893.7461	223.436	15.30
Residual evaporation regression formula	4	137.3554	34.339	2.35
Deviations from regression formulas	16	233.5717	14.598	
Total	24	1,264.6732		

Table 19 shows the amount of annual variance in yields accounted for by the rainfall and by the evaporation regression formulas. The mean square due to the rainfall regression formula when compared

with the mean square due to deviations from the regression formulas is highly significant.

In a similar comparison for the residual evaporation regression formula, an F' ratio of 2.35 is obtained. Although this value is less

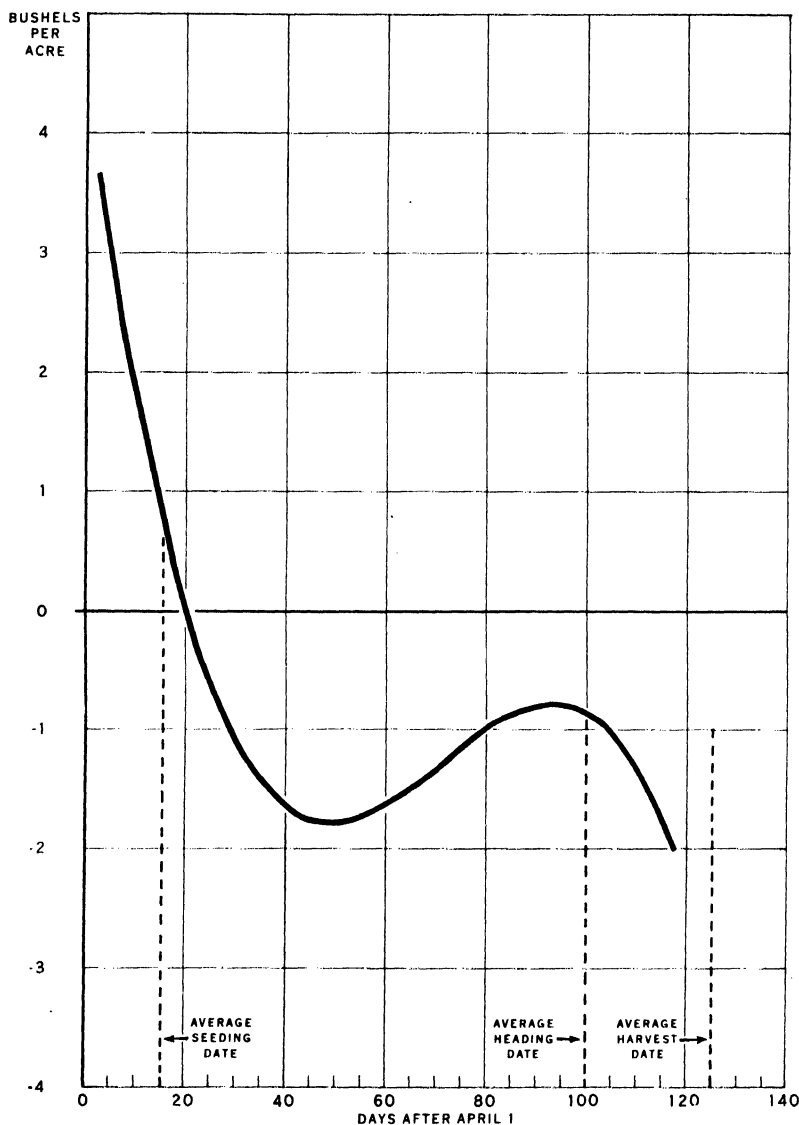


FIGURE 3.—Average effect of an additional inch of evaporation (influence of associated rainfall being eliminated) on yield of spring wheat from continuously cropped plots at Dickinson, N. Dak.

than the F' ratio of 3.01 at the usual 5-percent level of significance, it indicates that the effect of additional evaporation as shown in figure 3 may be real. Further support is contained in an unpublished study

of data from additional dry-land experiment stations in which the residual evaporation regression formula was found to be significant. It is therefore not probable that the effects of evaporation occur entirely by chance.

The partial regression coefficients of the various terms in the two regression formulas are shown in table 20 along with the probability

TABLE 20.—*Values and tests of significance of regression coefficients*

Degree	Rainfall regression formula		Evaporation regression formula	
	Regression coefficients	Probability	Regression coefficients	Probability
0	2.19723569	$P < 0.001$	-0.73867623	$0.03 < P < 0.05$
1	-.04157813	$0.2 < P < .3$	-.05797093	$.1 < P < .2$
2	-.00657100	$.05 < P < .1$.00616057	$.2 < P < .3$
3	-.00158232	$.01 < P < .02$	-.00084537	$.3 < P < .4$

of these occurring by chance. It should be noted that the partial regression of yield on the total evaporation for the season represented by the zero term of the formula is significant.

The coefficients of multiple correlation between yield and rainfall and between yield and rainfall and evaporation are represented by the values of R equals 0.82 and 0.90 respectively. /

DISCUSSION

The results for plot 5 of continuous corn grown at Wooster show clearly that although the total rainfall for the season is not significantly correlated with yield, the distribution of the seasonal rainfall is correlated with yield. In terms of the polynomials fitted to seasonal rainfall, it is the linear component that is significantly correlated with yield. This is evident from the general upward slope of the curve which indicates that for corn the effect on yield of an additional inch of rain increases with time over the period studied. In general, those familiar with the crop would expect this, although, so far as the writers are aware, no quantitative expression has heretofore been produced. As the crop progresses and the plants become larger with increased surface for transpiration, one would expect a favorable response to increased quantities of rainfall. Conversely, as may be interpreted from the function, in the period from planting to near maturity, the later in the season a deficit in rainfall occurs, the greater the detrimental effect. Frequently, adverse conditions early in the life of the corn plant are overcome as the season progresses. The fact, that, for the first one-third of the period covered, the curve extends below the zero line indicates that the mean rainfall at Wooster for this part of the season is greater than the optimum for corn.

Importance cannot be attached to fluctuations of the function a about its linear trend upward because, as pointed out in the analysis of the contributions of the several terms to total variation accounted for by the regression formula, none of the other terms has a significant partial regression coefficient. Bearing in mind the unimportance of the other terms of the regression formula, there exist, however,

agronomic facts of common knowledge that suggests the meaning of some of these fluctuations. During the early part of the life of the corn plant its requirement for moisture is limited to an amount sufficient for germination and the small degree of growth that takes place. This amount of moisture is seldom lacking at Wooster at this time. Furthermore, weeds must be kept from the corn, and to make this possible, the soil must be dry enough for cultivation. Any increased amount of rain hinders this. Wet weather is also conducive to weed growth. The amount of nitrogen available to the plant is thought to be enhanced by a warm aerated soil, a condition that cannot exist with large amounts of rainfall. Additional rainfall at this time may hinder root growth—not only the total amount of growth but also the character of it. If the weather is somewhat dry at this time, the roots will penetrate deeper into the soil and thus produce a condition that will better enable the plant to withstand dry weather later. These facts seem to lend support to the downward fluctuations of the curve immediately following the time of planting.

Once the plant is well started, with many of its structural parts laid down and freed from the competition of weeds, it rapidly elongates and responds to increasing amounts of rainfall, as is evidenced by the rapid rise in the curve. The peak of the curve is reached in about 80 days from the beginning of the season and occurs slightly earlier in relation to the average date of silking than might be expected. A great deal has been written concerning a critical period for crops at the time of pollination. Although the peak of the curve is not centered on this period, increased rainfall immediately preceding silking has a very beneficial effect and can be considered as placing the plant in a favorable condition for entering the reproductive stage. The position of the peak is determined somewhat by the character of the curve fitted, and the exact point where the effect of an additional inch of rainfall fails to exceed the effect in the period immediately preceding remains indeterminate. It should be emphasized that the tails of the curve, and even the points on the curve a slight distance from the tails, are regulated by the nature of the curve fitted and relatively little importance should be attached to the extremities of the curve.

The results of the study of the spring wheat crop grown in a more or less arid climate are in sharp contrast to those with corn grown in a humid climate. There is a high correlation between the total seasonal rainfall and yields of spring wheat, whereas in the case of corn the total seasonal rainfall has little association with yields. The average rate of increase or decrease in rainfall from the beginning to the end of the season is not significantly correlated with spring wheat yields, yet in the case of corn the results show that over the period studied the yields are higher if the rainfall increases at a constant rate during the season. Another contrast is that the parabolic and cubic terms in the rainfall sequence are important in the case of wheat but relatively unimportant in the case of corn. It is recognized that had the season been extended for corn the effect of additional rain would have diminished toward the time of harvest. Furthermore, the continuous corn series used may reflect a greater benefit from additional rainfall toward the end of the season than would corn on rotated

land. Nevertheless, it appears that for wheat the effect of additional rain rapidly diminishes from the time of heading. This appears to be an inherent biological characteristic of wheat.

The rainfall curve displayed in figure 2 would indicate that additional rainfall at any time during the growing season causes an increase in yield. The greatest beneficial effect is from rain that comes during the rapid growing period of the plant, reaching a maximum about 3 weeks before average heading date. At this time of the season each additional inch increases the yield about 4 bushels per acre.

The fact that these results indicate a rather definite period when additional rainfall has a much greater effect on yield than during the rest of the season, whereas Hopkins' results showed less evidence of this, needs consideration. Hopkins (5) recognized, however, that his results are not necessarily incompatible with the existence of a relatively critical period of growth by pointing out that any such period may be overshadowed by conditions of the soil and the amount of moisture present at the beginning of the season.

The problem of what degree polynomial to use in representing sequences of weather when studying spring wheat deserves comment at this point. As previously mentioned, the fourth and fifth terms in a polynomial fitted to seasonal rainfall at Dickinson were not significantly correlated with spring wheat yields. It would appear from the work of Hopkins (5) that in case of his Marquis series and rainfall, anything inclusive of the parabolic term would be adequate. The same is true for his stubble series, and here it is even highly questionable whether anything more than the total rainfall is correlated with yield. It appears, therefore, from this and the preceding paragraphs, that the quantity a , representing the average benefit to the spring wheat crop in bushels per acre per inch of rain falling in the time element considered, is a slowly changing function that can be easily represented by polynomials of the third degree or possibly lower.

It is concluded that, while yield appears significantly related to the total seasonal evaporation, the effect of the seasonal distribution of evaporation is not conclusively shown. However, the results are not unreasonable. Figure 3, showing the effect of the amount and distribution of evaporation on spring wheat yields, when associated rainfall effects are eliminated, indicates that above-average evaporation is detrimental to the crop over most of the growing season. Early in the season high evaporation, partly a result of above-average temperatures, would be favorable for obtaining a good stand of wheat. Later in the season, after the heading, increased evaporation may hasten ripening and cause a lowering of yield.

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DEVELOPMENT OF THE STAMINATE AND PISTILLATE INFLORESCENCES OF SWEET CORN¹

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INTRODUCTION

Studies of the morphology of the corn plant have led, according to Weatherwax (17),² to clearer and simpler explanations of the results of certain experiments with corn. It should be expected that further studies of the developmental morphology might prove to be equally helpful. Variations from the normal sequence of development which lead to abnormalities of the tassel and ear and variations in such characters as length of ear, number of rows of kernels on the ear, irregularities in the straightness of the rows of kernels, and correlation in the development of the ears of multiple-eared types, to mention a few examples, can be better understood by studying the developmental morphology. By means of frequent observations during the development of the growing points it is possible to see whether the variations observed in the mature plant are the result of variation in the pattern of differentiation and development or are the result of growth responses to changes in the environment.

While excellent descriptions of the morphological development of certain of the parts of the inflorescences have been written, some of which will be cited later, few workers have attempted to describe and illustrate all of the steps in the ontogeny of the staminate (tassel) and pistillate (ear) inflorescences. In this paper the major steps in the development of the tassel and ear from the undifferentiated growing points of the shoots to the fully differentiated flowers and flower parts are described and illustrated with photomicrographs.

LITERATURE REVIEW

Only a few of the publications dealing with the morphology of the corn plant will be reviewed. Most of the authors cited have made extensive reviews of the literature. In this connection special attention is called to the publications of Weatherwax (15), Miller (8), Randolph (11), and Arber (1).

The general morphology of the tassel and ear of the corn plant has been described by Collins (5), Weatherwax (15, 16, 18), and Arber (1). A clear understanding of the development of the pistillate spikelet is given by Miller (8), and Randolph (11) has described and illustrated the development of the pistillate spikelet and the caryopsis. Schuster (12) has described and illustrated some of the early stages of spikelet development.

Noguchi (9) is the only one of those cited who has described some of the beginning stages in the development of the ear and tassel, and he has illustrated a few of these stages with drawings.

¹ Received for publication May 22, 1939.

² Italic numbers in parentheses refer to Literature Cited. p. 36.

Descriptions of many-flowered spikelets, seeds in the tassel, and other variations from normal development in the inflorescences have been published by Kempton (6), Stratton (13), and Weatherwax (18).

MATERIALS AND METHODS

First-generation hybrids, Golden Cross Bantam (Purdue Bantam 39 \times Purdue 51) and a Country Gentleman hybrid (Illinois 8 \times Illinois 6) were used in these studies. Sweet corn (*Zea mays* var. *rugosa* Bonafous) was used because it could be easily grown in the greenhouse and, with an early-maturing type like Golden Cross Bantam, it was possible to get the various stages of development in a relatively short time. Another reason for using hybrids was that there would probably be less variation in development among individual plants which would make it easier to follow the developmental sequences.

In a study of the developmental morphology of the caryopsis, Randolph (11) found no significant differences among dent, flint, and sweet corn. Likewise, no essential differences were noted in the morphological characteristics of the inflorescences of dent and sweet corn. Therefore, it seems reasonable to expect that whatever is found out regarding the ontogeny of the inflorescences of sweet corn would also apply to dent corn.

The corn plants were grown in the greenhouse, without artificial lights, in glazed 2-gallon gars, filled with a mixture of sand, soil, and well-rotted stable manure.

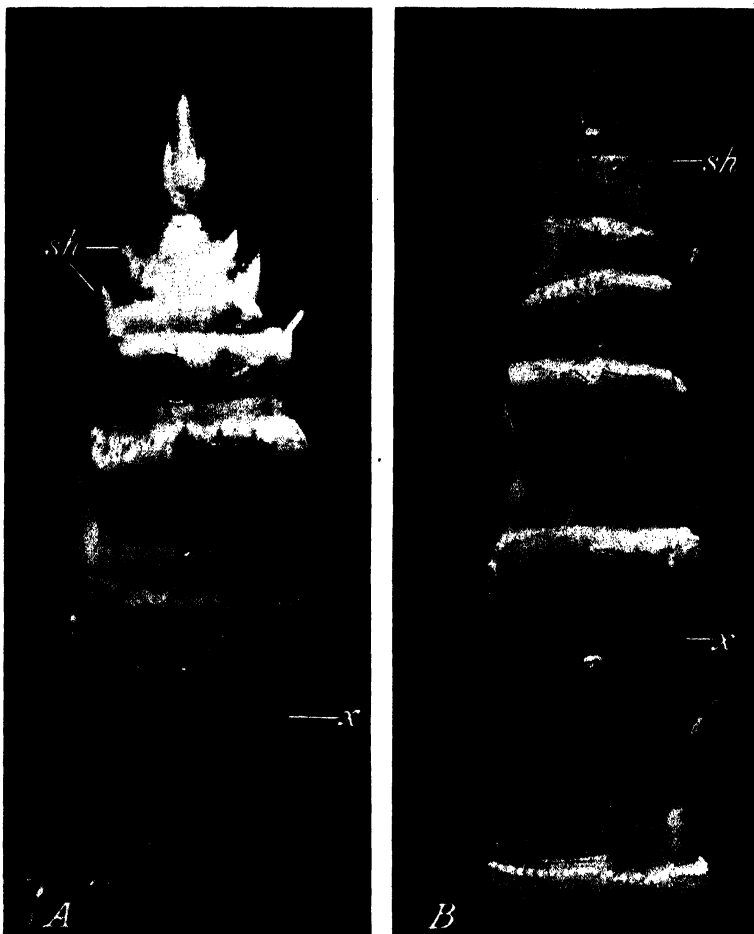
Growing points at successively later stages of development were removed from the plants and photomicrographs were taken. The photographic set-up was essentially the same as one already described (2). Photomicrographs were taken with an upright camera and special microlenses having focal distances of 16, 24, 32, and 48 mm. Light for photographing was obtained from a microscope lamp fitted with a 200-watt bulb. A Florence flask filled with distilled water was used as a condenser.

In order to bring out the morphological details in some of the specimens, a stain composed of a mixture of 90-percent alcohol, a small amount of glycerin, and basic fuchsin was applied. The alcohol quickly evaporated, leaving the stain and glycerin in the folds of the various structures which made them more easily seen.

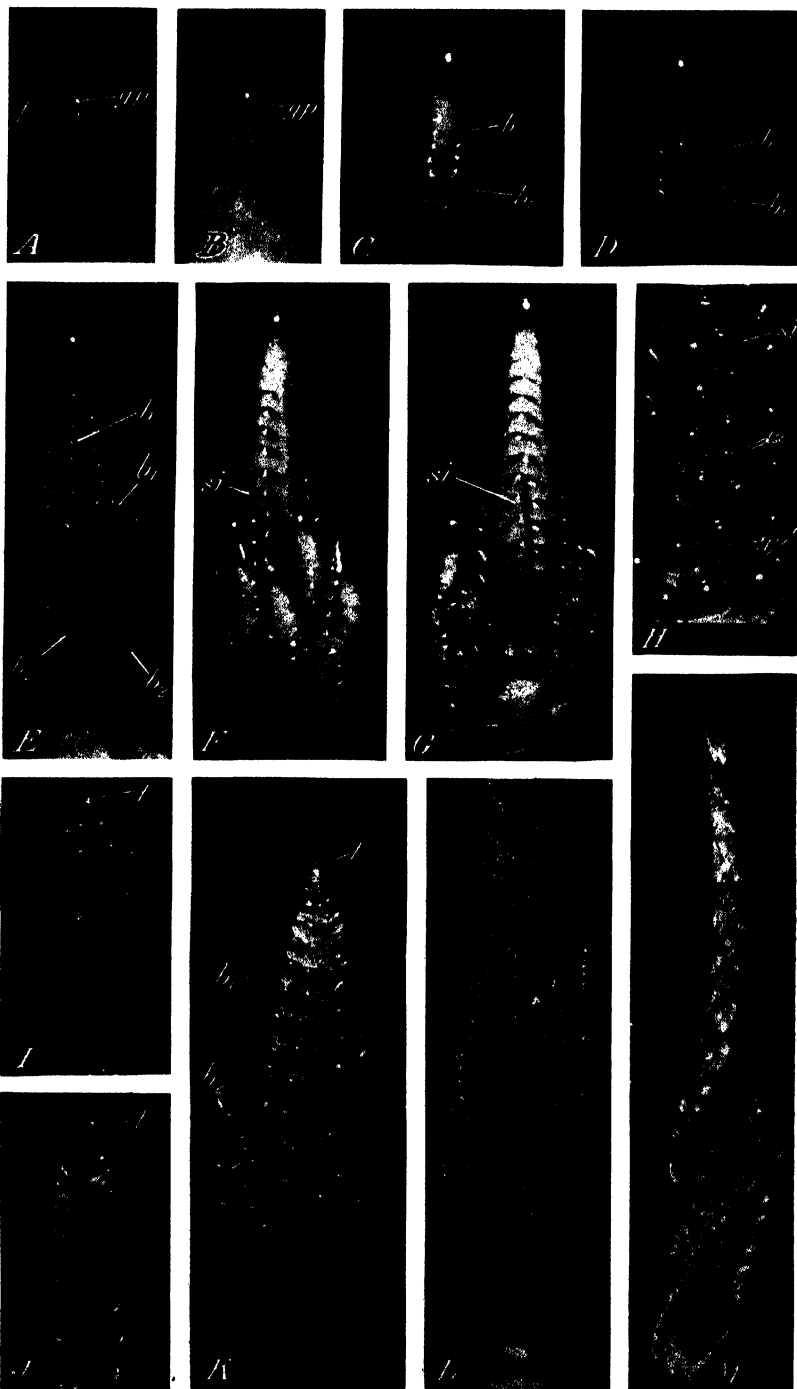
DEVELOPMENT OF THE INFLORESCENCES

Usually when the plants had 8 to 10 leaves, the ear shoot and tassel had begun to form (fig. 1). However, the number of leaves that a plant has is not a reliable guide to the stage of development of the inflorescence. Plants with the same number of leaves may differ to a considerable extent in the degree of development of the growing points owing to differences in growing conditions, differences in variety, and other factors.

Tassel and shoot development in a plant that was at the same stage of development as those shown in figure 1 is illustrated in plate 1, A. The tassel has formed, the axillary shoots from which the ears will develop (pl. 1, A, *sh*, and B, *sh*) can be seen on the upper part of the stem, and the basal internodes of the stem have begun to elongate (pl. 1, A, *x*, and B, *x*).



Stems of Golden Cross Bantam sweet corn with leaves removed to show the tassell, shoots, nodes, and internodes. *A* is an earlier stage than *B*; *x*, Internodes; *sh*, axillary shoot. $\times 5$.



For explanatory legend see opposite page.

TASSEL AND STAMINATE SPIKELET DEVELOPMENT

The shoot of the corn plant, like that of other cereals (2, 3, 4) and grasses (20), passes through two stages in its development from germination to the dehiscence of the anthers. During the first stage, leaf fundamentals, leaves, and axillary shoots are produced and the internodes of the stem remain short. During the second stage the internodes of the stem elongate, the tassel and its parts differentiate and develop, and the axillary shoot or shoots (ear or sucker) pass through their various stages of development.

Two growing points (pl. 2, *A* and *B*) represent the appearance of the shoot in the first stage of development. The growing point (pl. 2, *A*, *gp*) is partly enclosed by two leaf initials. At this stage of development the growing point is much smaller in relation to the diameter of the stem than the growing point of either wheat, oats, or barley at a similar stage of development.

Two processes, which occur simultaneously, indicate the beginning of the second stage of development. (1), The internodes of the stem begin to elongate (pl. 1, *A*, *x*, and *B*, *x*), and (2) the growing point elongates in preparation for the differentiation of the tassel and its parts. During the second stage the growth activities are internode elongation and the differentiation of the tassel and its parts. Tassel development is completed when the anthers dehisce.

Branch primordia are the first of the tassel parts to differentiate (pl. 2, *C*, *b*, and *D*, *b*). They arise in acropetal succession as lateral projections from all sides of the elongated central axis. Some of the branch initials at the base of the central axis elongate and become the lateral axes of the tassel (pl. 2, *E*, *b*₁). The other initials arising from a point higher on the central axis are the initials from which the spikelet initials originate (pl. 2, *C*, *b*; *D*, *b*; *E*, *b*; *F*, *si* and *G*, *si*).

It should be noted that, in the early stages of development of the tassel, so far as external appearances indicate, there are no differences between those initials that become the lateral branches of the first order and those from which the spikelet initials differentiate. Therefore all of the first initials to appear are branch initials.

EXPLANATORY LEGEND FOR PLATE 2

A.—Growing point of a corn plant having four leaves visible. × 22.

B.—Beginning of the elongation of the growing point just before tassel differentiation. × 22.

C and *D*.—Differentiation of the branches of the tassel. × 22.

E.—Elongation of the basal branches of the tassel. × 22.

F.—Beginning of the differentiation of the spikelet initials on the central axis of the tassel. × 22.

G.—A stage similar to *F* with some of the basal branches removed to show spikelet differentiation on the central axis. × 22.

H.—Differentiation of spikelets and empty glumes on a portion of the central axis of the tassel. × 35.

I.—Portion of the tip of the central axis of the tassel. × 25.

J.—Adaxial side of a branch of the tassel. × 22.

K.—Abaxial side of tassel branches of the first and second orders. × 25.

L.—A more advanced stage of tassel development. × 22.

M.—Fully differentiated but not full-sized tassel. × 8.

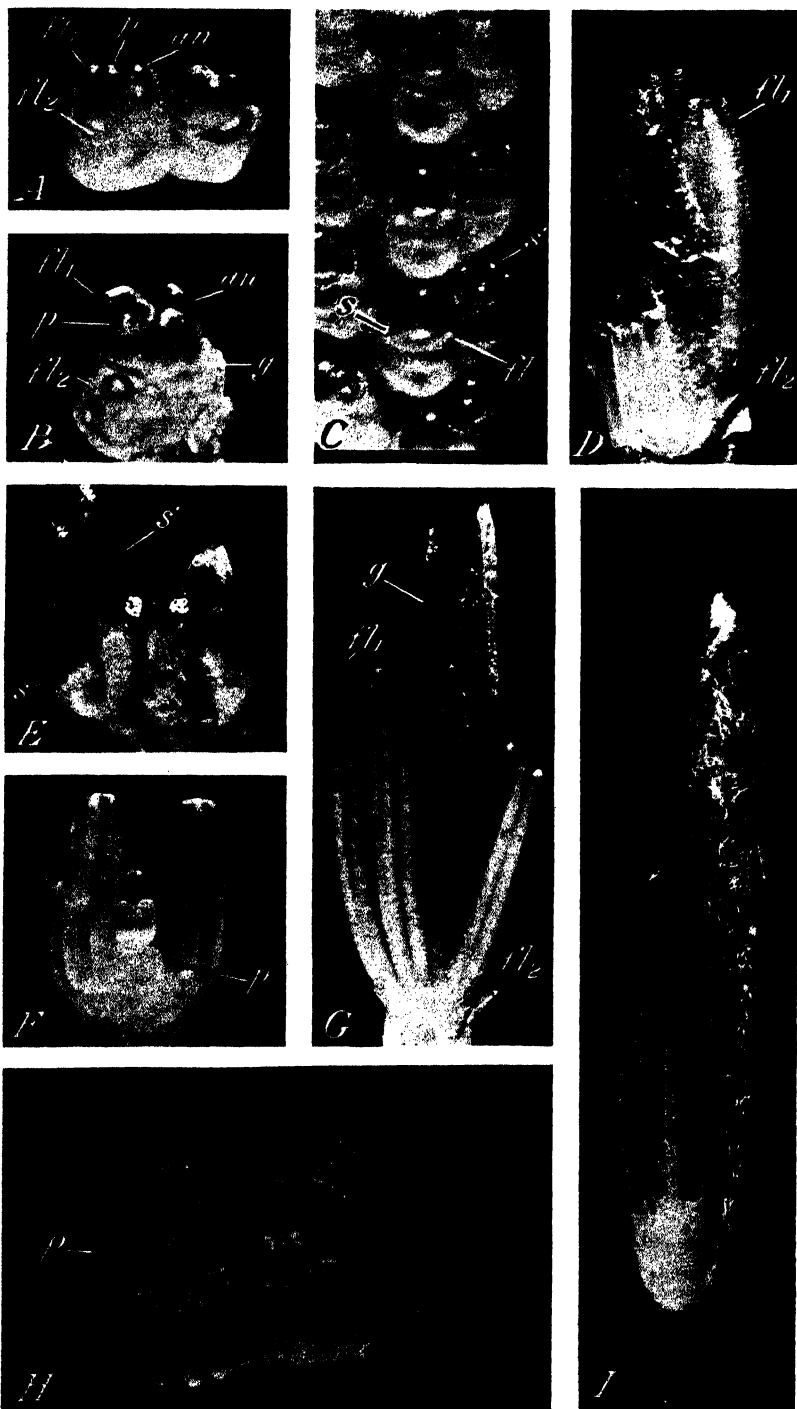
b, Branch initial from which spikelets differentiate; *b*₁, basal branch of the first order; *b*₂, branch of the second order; *e*, empty glumes; *gp*, growing point; *gp'*, growing point of the spikelet; *l*, leaf initial; *si*, spikelet initial; *t*, undifferentiated tip of an axis.



FIGURE 1.—Plants of Golden Cross Bantam sweet corn at the same stage of development as the dissected plants shown in plate 1, A and B.

As has been described for oats (4), branches of the second order may rise by budding from the base, and at the lateral margins, of the branches of the first order (pl. 2, *E*, *b*, and *K*, *b*). As has already been stated for the central axis, those initials of the lateral axes above the most basal ones are the primordia from which the spikelet initials differentiate.

In studies made on barley, wheat, and oats (2, 3, 4) there was always an indication of leaf fundamentals on the central axis in the axils in which the lateral shoots of the inflorescence were formed. There was, however, no indication of leaf fundamentals subtending the initials.



For explanatory legend see opposite page.

of the lateral shoots of the tassel, but, as will be pointed out later, there are structures apparently homologous with leaf initials, sub-ending the lateral shoots of the ear (pl. 4, *C, l*).

All the branches of the tassel are indeterminate. Neither the central axis (pl. 2, *I, t*) nor the lateral axes (pl. 2, *J, t*, and *K, t*) of the tassel terminate in apical spikelets. Primordia from which the spikelet initials differentiate are produced acropetally as long as the axes increase in length.

In the beginning of spikelet development the branch initial divides into two unequal parts, the spikelet initials (pl. 2, *F, si*; *G, si*; and *H, si*). The spikelet that develops from the larger spikelet initial is pediceled (pl. 3, *E, s'*) and the spikelet from the smaller spikelet initial is sessile (pl. 3, *E, s*). The larger initial is always in advance of the smaller in its development. This is shown by the beginning of development of the empty glume on the larger initial in plate 2, *H, e*, and the lack of such development in the smaller initial and by the beginning of anther differentiation in the larger spikelet in plate 3, *C, s'*, and the lack of anthers in the smaller spikelet plate 3, *C, s*.

Several of the early stages of development of the spikelets can be seen in plate 2, *H*, which shows a group of spikelets from the central axis of the tassel. The stages of development beginning at the top of the photograph range from an undifferentiated lateral shoot initial, through the various stages of division into spikelet initials, to the beginning of development of the empty glumes. The empty glumes are the first of the spikelet parts to form and are first seen as transverse ridges across the spikelet initial (pl. 2, *H, e*). They grow in length and finally enclose the flowers (pl. 3, *I*).

Spikelet initials develop from all sides of the central axis of the tassel (pl. 2, *G*) but only on the abaxial side of the lateral branches. The abaxial side of branches of the first and second order are shown in plate 2, *K, b₁*, *b₂*, and the adaxial side of a branch is shown in plate 2, *J*. Two rows of lateral shoot primordia develop and they divide into two pairs of spikelet initials.

At any stage of development the central axis of the tassel is in advance of the branches (pl. 2, *F, L*, and *M*). This is what should be expected since the central axis is formed first and the branches

EXPLANATORY LEGEND FOR PLATE 3

A.—Two spikelets of the tassel at the beginning of the development of the flower parts of the upper flowers and the more advanced stage of development of the spikelet at the left. × 56.

B.—Two flowers of a spikelet of the tassel showing the more advanced stage of development of the upper flower. × 56.

C.—Part of a branch of the tassel showing a more advanced stage of development of the pediceled spikelet. × 40.

D.—Staminate spikelet with the empty glumes removed to show the difference in the size of the anthers of the upper and lower flower. × 20.

E.—Two pairs of spikelets, one member of each pair is pediceled and the other sessile, the empty glumes have been removed from one spikelet. × 25.

F.—Staminate flower with one anther removed to show the partly developed pistil. × 20.

G.—Later stage of spikelet development in which the anthers of the lower flower are approaching the size of those of the upper flower. × 10.

H.—Pair of spikelets both sessile. × 28.

I.—Fully differentiated spikelet. × 10.

an, Anther; fl, flower initial; fl₁, upper flower; fl₂, lower flower; g, palea; p, pistil; s, sessile spikelet; s', pediceled spikelet.

differentiate from it. The branches increase considerably in size before the initials from which the spikelets differentiate are produced.

In each staminate spikelet two flowers develop from the meristem located above the empty glume initials (pl. 2, *H*, *gp'*). The meristem divides into two unequal parts. The larger part gives rise to the upper flower (pl. 3, *A*, *fl*₁, and *B*, *fl*₁) and the smaller part develops into the lower flower (pl. 3, *A*, *fl*₂, and *B*, *fl*₂). These flowers differ in their rates of development. The anthers of the upper flower (pl. 3, *A*, *fl*₁; *B*, *fl*₁; *D*, *fl*₁; and *G*, *fl*₁) differentiate first and in their development are always ahead of the corresponding parts of the lower flower (pl. 3, *A*, *fl*₂; *B*, *fl*₂; *D*, *fl*₂; and *G*, *fl*₂). As the flowers approach maturity the anthers of the lower flower attain nearly the same size as the anthers of the upper flower (pl. 3, *G*, *fl*₁, and *fl*₂).

Anther initials are the first of the flower parts to differentiate (pl. 3, *A*, *an*, and *B*, *an*). Since the tassel flowers are staminate, anther differentiation and development are the principal growth activities within the flower.

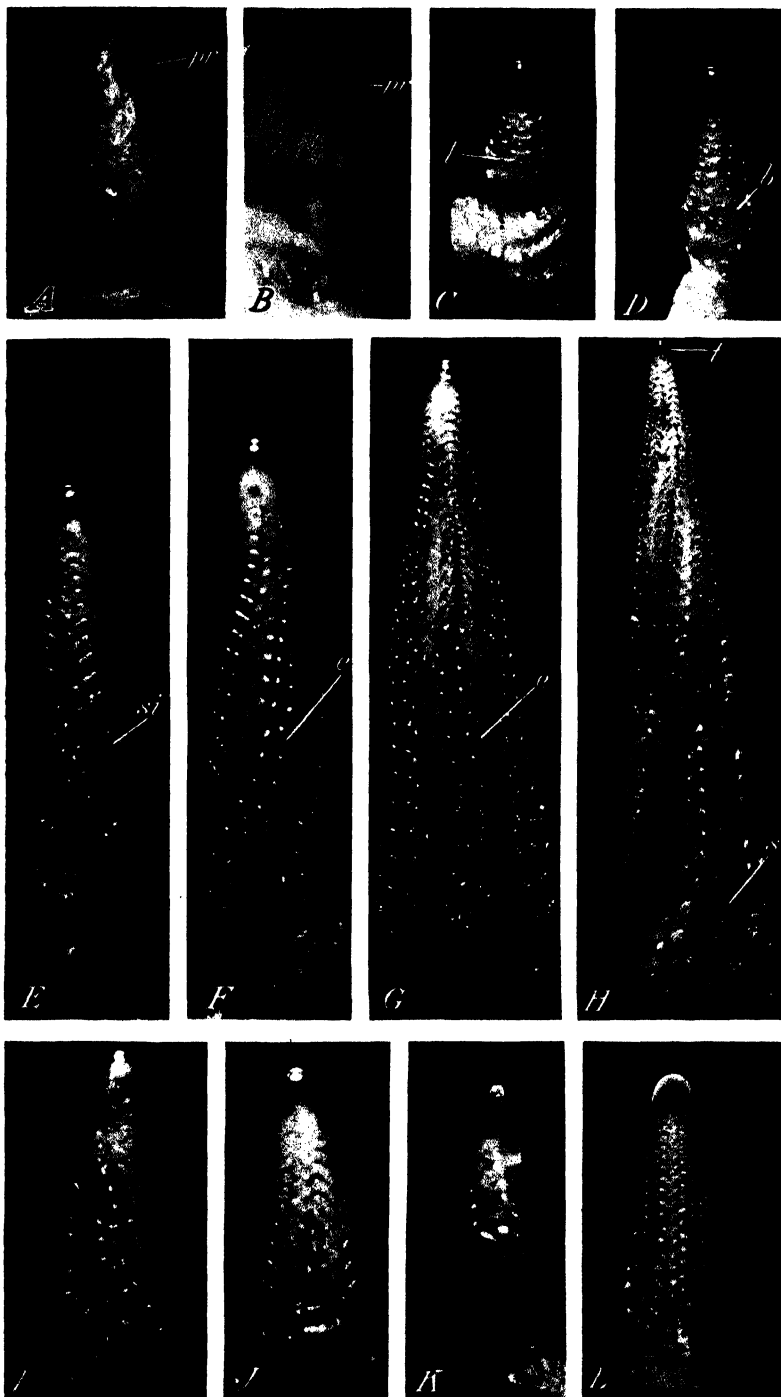
Pistils may develop from the meristem located above the anther initials (pl. 3, *A*, *p*) but they usually remain rudimentary (pl. 3, *B*, *p*). Under certain conditions of growth the pistil may show considerable development (pl. 3, *F*, *p*) and may become fully developed and functional (pl. 7, *E*, *p*).

Flowering glumes develop for each flower, but they are so thin (pl. 3, *G*, *g*) that they are difficult to distinguish at the beginning of their development. The lemma and palea begin their development as thin ridges at a point on the meristem just below the anther initials (pl. 3, *B*, *g*) at about the same time that the anther initials begin to differentiate.

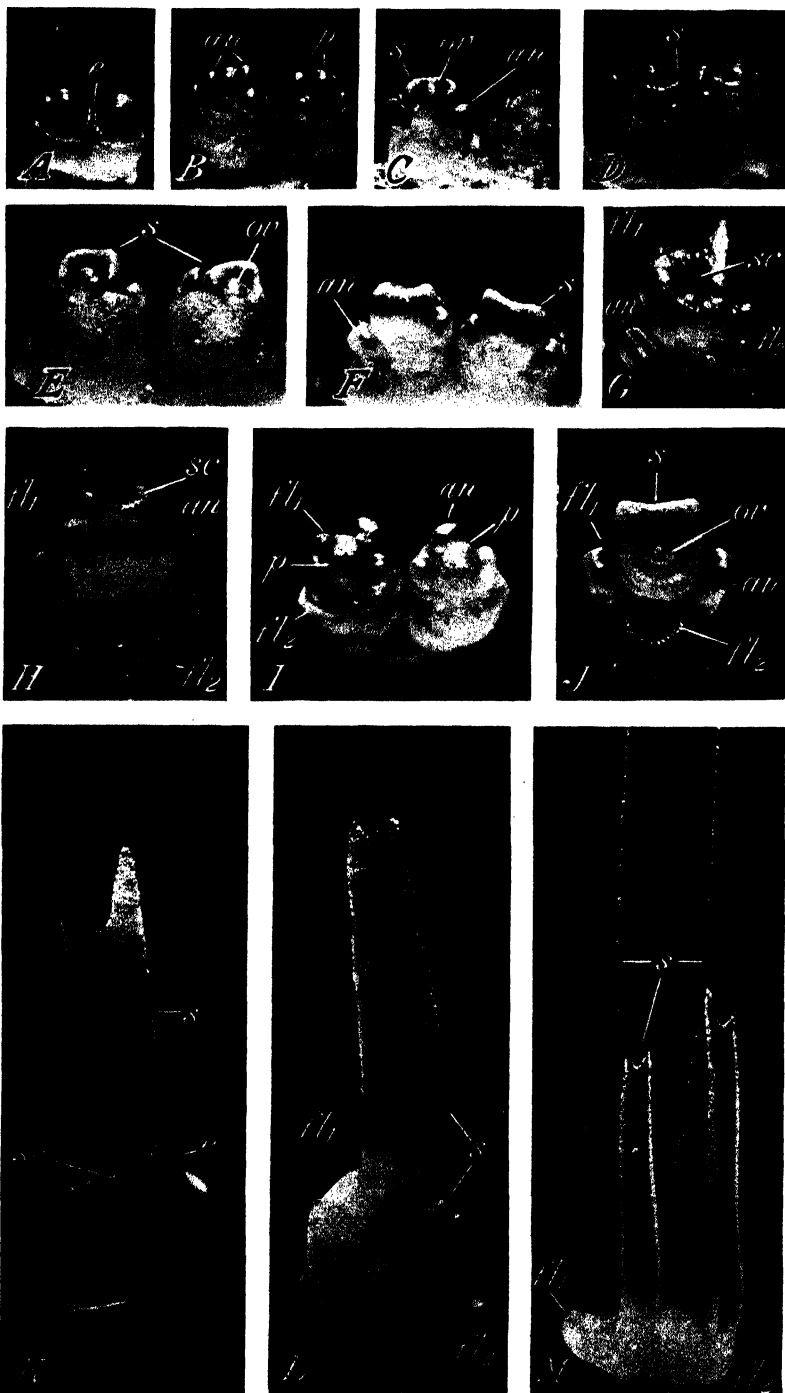
Deviations from the normal development under field conditions are often seen in plants grown in the greenhouse. Normally one spikelet is sessile and the other pediceled (pl. 3, *E*), but both spikelets may be sessile (pl. 3, *II*). Another type of deviation which will be described later is the development of functional pistils in the tassel.

EXPLANATORY LEGEND FOR PLATE 4

- A*.—Axillary shoot in which the ear develops, enclosed in the prophyllum. × 13.
- B*.—Side view of an axillary shoot. × 17.
- C*.—Beginning of the differentiation of the ear. × 25.
- D*.—Ear development showing a more advanced stage of branch differentiation. × 25.
- E*.—Beginning of spikelet differentiation by an unequal division of the branch initials. × 22.
- F*.—Development of the empty glumes. × 22.
- G*.—Paired rows of the ear and a more advanced stage of the development of the empty glumes. × 17.
- H*.—The differentiation and development of the silks can be seen at the base of the ear. × 17.
- I*, *J*, and *K*.—Topmost, second, and third ears, respectively. All × 22.
- L*.—Young ear of Country Gentleman sweet corn. × 17.
- b*, Branch initial from which spikelet initials differentiate; *e*, empty glumes; *l*, leaf fundament; *pr*, prophyllum; *st*, spikelet initial; *s*, silk initial; *t*, undifferentiated tip of the ear.



For explanatory legend see opposite page.



For explanatory legend see opposite page.

EAR AND PISTILLATE SPIKELET DEVELOPMENT

In the early stage of stem development a shoot is produced in the axil of each leaf (pl. 1, *A*, *sh*, and *B*, *sh*), but at a later stage of development axillary shoots are no longer produced. The cessation of axillary shoot development seems to be associated with the elongation of the internodes of the stem and the development of the tassel. This is in agreement with observations made by Percival (10) on the cessation of axillary shoot (tiller) development in wheat and the same thing has been observed regarding axillary shoot development in barley.³

Ears develop from the upper one or more axillary shoots of the stem. Those shoots formed at the base of the stem may remain nonfunctional or develop into suckers. If an examination is made at the time the topmost shoots are producing ear initials, it will be found that the growing points of the basal shoots are producing only leaf fundamentals; but they are more and more advanced in development from the base to the top of the stem.

Axillary shoots develop in acropetal succession and during the early stage of stem development the axillary shoots became larger in succession from the apex to the base of the stem (pl. 1, *A*). Later when the ears begin to develop, the size sequence changes, so that the topmost shoot is the largest and the shoots become smaller from the top to the base of the plant (pl. 1, *B*). The topmost shoot or the topmost two or three shoots, depending upon whether they are single- or multiple-eared types, in turn take precedence in their development or they may inhibit the development of the shoot immediately below. This difference in development is shown by the size of the ear initials in plate 4, *I*, *J*, and *K*, which are the ear initials from the topmost, second, and third shoots, respectively.

³ BONNETT, O. T. TILLERING IN BARLEY AS INFLUENCED BY CERTAIN PLANT CHARACTERISTICS. 1933. (Abstract of doctor's thesis, Univ. Ill.)

EXPLANATORY LEGEND FOR PLATE 5

- A*. Pair of pistillate spikelets at an early stage of development. $\times 40$.
 - B*. Beginning of the differentiation of anthers in the upper flower of a pair of spikelets. $\times 40$.
 - C*.—Differentiation of a silk, the first stage of pistil development. $\times 40$.
 - D*. Silk development. $\times 40$.
 - E*.—Silks partly enclosing the ovules. $\times 40$.
 - F*.—Silk development from the adaxial side. $\times 40$.
 - G*.—Spikelet showing the comparative development of the upper and lower flowers. $\times 40$.
 - H*.—Functional upper flower and a sterile lower flower of the spikelet are illustrated. $\times 40$.
 - I*.—Pair of spikelets of Country Gentleman at an early stage of development. $\times 40$.
 - J*.—Spikelet of County Gentleman comparing the development of the functional upper and lower flowers. $\times 40$.
 - K*.—Silk development of the functional flower of a spikelet having only one functional flower. $\times 28$.
 - L*.—Comparison of the silk development of the lower and upper flowers of a spikelet having two functional flowers. $\times 28$.
 - M*.—A more advanced stage in the development of spikelets having two functional flowers. $\times 10$.
- an*, Anther initial; *e*, empty glumes; *f*₁, upper flower; *f*₂, lower flower; *ov*, ovule; *p*, pistil initial; *s*, silk initial; *sc*, stylar canal.

The axillary shoot is enclosed in a strongly keeled prophyllum (pl. 4, *A, pr*, and *B, pr*) which may be entire or divided. Leaf initials that develop into the husks are covered by the prophyllum.

Ear differentiation is indicated by an elongation of the growing point of the axillary shoot and the differentiation of lateral projections from the central axis of the ear initial (pl. 4, *C*, and *D*). The lateral projections are the initials from which the spikelet initials differentiate and correspond to the initials that first appear on the central axis and branches of the tassel. Subtending each initial, as has already been mentioned, are ridges (pl. 4, *C, l*) which are similar to the subtending leaf initials that appear in the differentiation of the inflorescences of barley, oats, and wheat. These ridges increase in size and form the cuplike depressions in which the spikelets occur (pl. 6, *B, x*).

Spikelet initials are produced in pairs by the division of the preceding initial into two unequal parts (pl. 4, *E, si*). While the parts of the larger of the pair of spikelet initials begin to differentiate before those of the smaller spikelet initial, the difference in their development is not so great as was pointed out for the spikelet initials of the tassel.

The empty glumes are the first of the spikelet parts to form and can be seen as transverse ridges across the spikelet initial (pls. 4, *F, e*, and 5, *A, e*). More advanced stages of development of the glumes are shown in plate 4, *G, e*. As the empty glumes increase in length, they enclose the ovary, but the silk extends beyond them (pl. 5, *K, e*).

Straightness of row and the number of rows of kernels per ear are characteristics of the ear that are determined when the spikelets differentiate. Variations in the straightness of row can be seen in plate 4, *E, F, G*, and *H*. The rotation to the left or right (pl. 4, *E* and *F*) and the regularity or irregularity in the placement of the spikelets (pl. 4, *G* and *H*) can be seen. Row number is determined by the number of rows of branch initials around the ear initial (pl. 4, *D, b*) from which a pair of spikelets differentiates. Each spikelet has a fertile flower from which the kernels are produced.

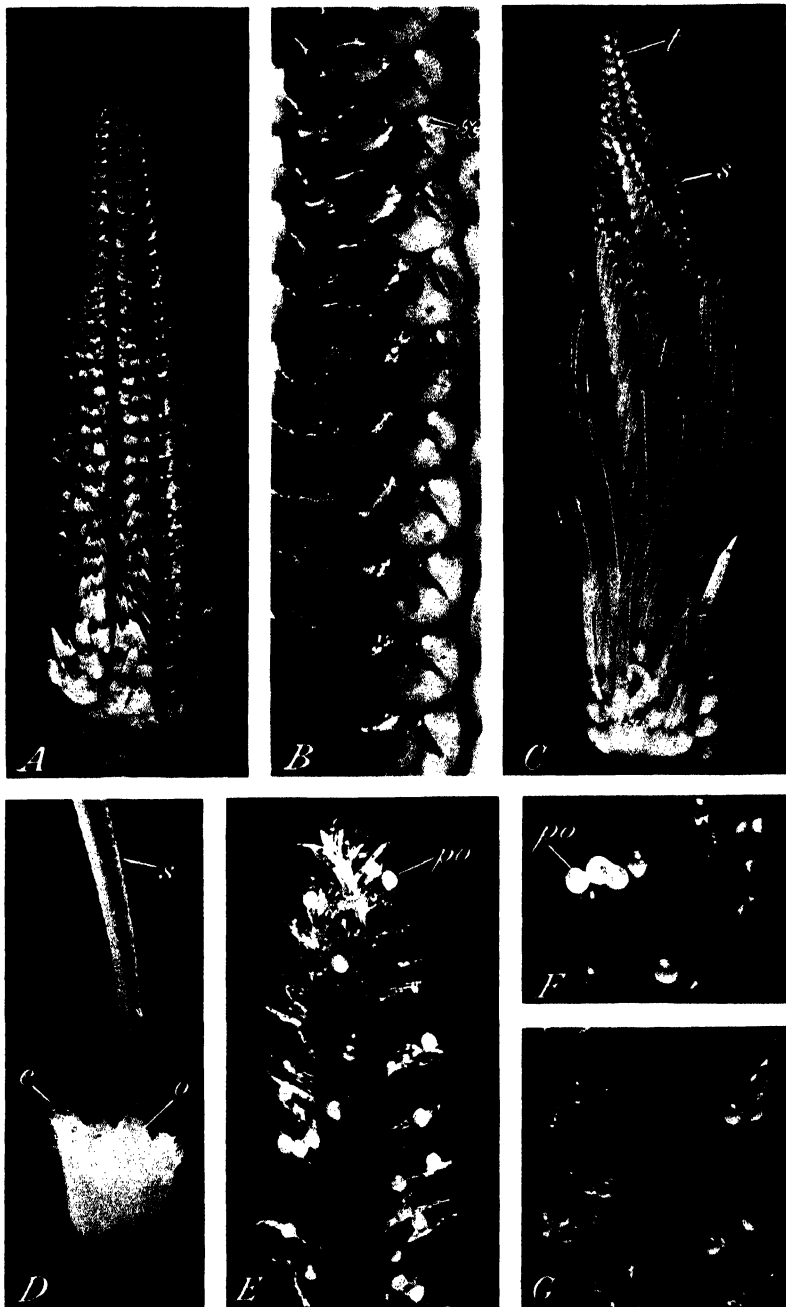
The ear as well as the tassel is indeterminate in its growth and continues to elongate at the tip (pl. 4, *H, t*), but many of the flowers at the tip of the ear remain rudimentary (pl. 6, *C, t*). Since the spikelets arise in acropetal succession they are successively younger from the base to the tip of the ear (pl. 4, *G* and *H*).

Two flower initials are produced in each spikelet, but in most corn varieties only one flower is functional. In a few types like Country Gentleman sweet corn both flowers are functional.

The two flowers of the ear develop from an unequal division of the meristem of the spikelet just as was pointed out for the flowers of the tassel. The flower differentiating from the larger mass of meristem

EXPLANATORY LEGEND FOR PLATE 6

- A*.—Ear showing different stages of silk development. × 6.5.
- B*.—Section of an ear. × 19.
- C*.—Variation in silk development. × 6.5.
- D*.—Pistil from the tassel. × 14.
- E*.—Tip of a mature silk with pollen grains germinating on it. × 26.
- F* and *G*.—Pollen grains germinating on the silk. × 38.
- e*, Empty glumes; *o*, ovary; *po*, pollen grain; *s*, silk; *t*, tip of ear; *x*, enlargement of ridge subtending the spikelets of the ear.



For explanatory legend see opposite page.



For explanatory legend see opposite page.

(the upper flower) takes precedence in its development over the flower from the smaller mass of meristem (the lower flower). The larger flower is the functional flower in those types of corn that have only one functional pistillate flower per spikelet. In these types of corn having two functional pistillate flowers per spikelet, the larger flower is more advanced at every stage in its development than the smaller flower.

Anther initials are the first of the reproductive parts of the flower to differentiate (pl. 5, *B*, *an*). In the pistillate flower the anthers begin differentiation but usually remain small and nonfunctional. Under certain growth conditions and in the genetic type, anther ear, the anthers of the pistillate flower may attain full development. Anthers well enough developed to show the locules are shown in plate 5, *G*, *an*, and *J*, *an*.

The pistil initial develops from the apex of the growing point which is located between the anther initials (pl. 5, *B*, *p*, and *I*, *p*). Development begins with the formation of a ridge, the silk initial, which partly encircles the tip of the growing point (pl. 5, *C*, *s*, and *D*, *s*). The ovule differentiates from the meristem which is partly enclosed by the developing silk initial (pl. 5, *C*, *ov*, and *E*, *ov*).

The margin of the silk initial grows more rapidly on one side than on the other (pl. 5, *E*, *s*). Soon two distinct points appear (pl. 5, *F* and *G*) which continue to elongate (pl. 5, *G* and *K*) and which finally result in the biparted tip of the mature silk (pl. 6, *E*). Unequal growth rates of the margins of the silk initial result in the development of a tubelike structure, partly enclosing the ovule (pl. 5, *E*, *ov*). The opening above the ovule gradually closes and becomes the stylar canal (pl. 5, *G*, *sc*; *H*, *sc*; and *K*, *sc*).

As the silk elongates it becomes covered with hairs, the structure of which has been described by Weatherwax (16). Hairs are just beginning to appear as fine points upon the silk in plate 5, *K*, *s*, and *L*, *s*, and they are shown, fully developed, with pollen grains germinating upon them, in plate 6, *E*, *F*, and *G*.

The ovary is shown in plate 6, *D*, *o*, with the silk attached and partly enclosed by the flowering glumes. At this stage of pistil development all of the external parts have differentiated but the pistil has not attained full size.

Silks begin to develop first at the base of the ear (pl. 6, *A*), and at later stages of ear development a marked contrast in the length of the silks at the tip and the base of the ear can be seen (pl. 6, *C*).

EXPLANATORY LEGEND FOR PLATE 7

- A*.—A staminate (left) and pistillate (right) spikelet from the tassel. $\times 20$.
B.—Silk development in a pistillate spikelet from the tassel. $\times 28$.
C.—Glumes removed from the pistillate spikelet to show the abortive lower flower. $\times 20$.
D.—Staminate and pistillate spikelets of the tassel. $\times 15$.
E.—A fully differentiated staminate and a pistillate spikelet of the tassel, the pistillate spikelet being sessile and the staminate spikelet pedicled. $\times 10$.
F.—A tassel showing silks from pistillate spikelets at the base of the tassel. $\times 10$.
f ₁, Upper flower; *f* ₂, lower flower; *p*, pistil; *s*, silk; *x*, upper flower of the staminate spikelet.

An enlargement of a section of the ear at the same stage of development as the ear in plate 6, *A*, is shown in plate 6, *B*. The attachment of the spikelets, variation in the length of the silks, and the size of the stylar canal can all be seen in plate 6, *B*.

Very soon after pollen grains lodge upon the silk they germinate and the pollen tube grows down the hair into the silk (pl. 6, *F* and *G*). This process has been described by Miller (8) and Randolph (9).

DEVIATIONS FROM NORMAL FLOWER DEVELOPMENT

Two deviations from normal flower development will be illustrated and described. The first is the development of two fertile flowers in a pistillate spikelet and the second is the development of pistillate spikelets in the tassel. Illustrations for the first deviation were taken from Country Gentleman sweet corn and are shown in plate 5, *I*, *J*, *L*, and *M*. Illustrations for the second deviation were taken from Golden Cross Bantam and are shown in plate 7.

It will be recalled that in those types of corn that have only one fertile flower per spikelet, the sterile flower begins but does not complete its development. The sterile flower develops from the smaller of the two divisions of the growing point of the spikelet initial. Anther initials and the pistil initial of the sterile flower differentiate (pl. 5, *G*, f_2) but do not complete their development (pl. 5, *II*, f_2). The pistil of the fertile flower develops as has been described, but the anthers do not, so that in examining a spikelet of the ear of those types of corn having one fertile flower per spikelet, all that can be seen after the silk has begun to elongate are the empty glumes (pl. 5, *K*, *e*) and the silk of the fertile flower extending beyond them (pl. 5, *K*, *s*).

When two fertile flowers develop in a pistillate spikelet each flower goes through the same sequence of development that has been described, but the rates of development are different. The upper flower arising from the larger of the two divisions of the growing point develops more rapidly than the lower flower. This was also pointed out for the development of the two flowers of the spikelet in the tassel.

The differences in rates of development of the upper and lower flowers can be seen by comparing the development of the upper flower in plate 5, *I*, f_1 ; *J*, f_1 ; *L*, f_1 ; and *M*, f_1 , with the development of the lower flower designated as f_2 in the photographs just mentioned. While the upper flower develops first, the lower flower gradually overtakes the upper flower as the ear approaches maturity, so that at pollination, the silks of both flowers are approximately the same length.

Paired grains of corn result when two fertile flowers are produced per spikelet. The germ of the upper flower faces the tip of the ear and the germ of the lower flower faces the base of the ear, resulting in the kernels being placed back to back. With the development of two grains per spikelet the kernels may be crowded out of line so that there are irregular rows or a lack of rows as shown in Country Gentleman sweet corn.

Development of paired grains, according to Randolph (11), was first described in pod corn by Sturtevant (14), and Kempton (6) was the first to interpret correctly the development of paired grains as being the result of the development of two fertile flowers per spikelet. Weatherwax (18), Stratton (13), and others have also described the development of double kernels.

The development of pistillate spikelets in the tassel is an interesting deviation from normal development. Kempton (6) has pointed out that if there are pistillate flowers in the staminate inflorescence, it is the upper flower of the sessile spikelet that is pistillate and both of the flowers of the pediceled spikelet are staminate.

Spikelet differentiation and the first stages in the development of the flowers are the same in both the staminate and the pistillate spikelets of the tassel. The essential difference lies in the degree of development of the anthers and pistils. In the pistillate flowers both the anthers and the pistil differentiate but the pistil takes precedence in development; in the staminate flowers both the anthers and pistil differentiate but the anthers develop instead of the pistil.

In Golden Cross Bantam only one flower, the upper one, of the spikelets of the ear is fertile and the same is true of the pistillate spikelet produced in the tassel. Consistent with the development of the flowers of the ear of this type, the upper flower (pl. 7, *A*, f_1 , and *C*, f_1) developed and the lower flower was abortive (pl. 7, *A*, f_2 , and *C*, f_2).

Pistil differentiation and development were the same as previously described for the pistillate flower of the ear. The various stages in the development of the silk are shown in plate 7, *A*, *s*, to *F*, *s*, inclusive, and it can be seen that they are essentially the same as already described.

The development of the flowers of the staminate spikelet shows no deviation from normal development except that the pistil is a little further developed than in the tassels having only staminate spikelets. But the example shown in plate 7, *A*, *x*, should not, perhaps, be considered as typical because even in those plants that did not have pistillate spikelets, a considerably greater degree of pistil development was noted (pl. 3 *F*, *p*, and *H*, *p*) than would be expected in plants grown in the field. However, this is what should be expected of corn plants grown in the greenhouse under certain conditions of temperature and light.

SUMMARY

The developmental morphology of the tassel, the ear, and their parts were studied by dissecting them from the stem of the corn plant at the different stages of development. Photomicrographs were taken of the various stages.

From germination to the dehiscence of the anthers, the shoot of the corn plant passes through two stages of development. In the first stage leaves and axillary shoots are produced, and in the second stage the internodes of the stem elongate and the tassel, ear, and their parts differentiate and develop.

Tassel differentiation begins with the appearance of lateral projections, branch initials, which arise acropetally from the growing point of the central axis. The first initials to appear at the base of the central axis elongate to produce branches of the first order. Those above develop into two spikelet initials.

Branches of the second order arise as buds from the base and at the margins of the branches of the first order.

Differentiation of the ear also begins with the appearance of lateral projections which arise acropetally from the growing point.

In both the tassel and ear the spikelet-forming branch initials divide into two unequal parts to form the spikelet initials and in turn the spikelet initials divide into two unequal parts to form the flower initials.

In the tassel the spikelet developing from the larger division of the branch initial is pediceled and the spikelet from the smaller division is sessile. In the ear and tassel the larger initial begins the development of its parts ahead of the smaller initial.

Differences in the size of the flower initials in the tassel are correlated with a difference in the size and rate of development of the anthers. The larger (upper) flower initial is ahead of, and larger than, the anthers of the smaller (lower) flower initials, but as the flowers approach maturity the anthers of the lower flower are almost as large as the anthers of the upper flower.

In the ear the flower developing from the larger (upper) of the two flower initials becomes the fertile flower and the smaller (lower) flower initial the abortive flower in those types of corn that have only one fertile flower per spikelet. In those types that have two fertile flowers per spikelet the flower from the upper initial is larger and develops before the flower from the lower initial.

The empty glumes are the first of the spikelet parts to differentiate in the spikelets of the tassel and the ear.

Flower parts of the flower of the tassel and of the ear differentiate in the following order: Lemma and palea, anthers, and pistil. In the pistil the ovary, silk, and hairs on the silk develop in the order named.

Pistillate spikelets which develop in the tassel follow the same sequence in their development as the pistillate spikelets of the ear.

When two fertile flowers develop in the pistillate spikelet of the ear both flowers follow the normal sequence of development, but the upper flower develops ahead of the lower flower.

Because they do not terminate in apical spikelets the ear and tassel are indeterminate inflorescence.

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THE DIGESTIBILITY OF MATURE RANGE GRASSES AND RANGE MIXTURES FED ALONE AND WITH SUPPLEMENTS¹

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INTRODUCTION

Bunch grasses have long been prized for their feeding value when mature. Where weather conditions permit their use, these grasses have been considered excellent winter feed for horses, cattle, and sheep.

The study herein reported was made to determine: (1) The digestibility of bluebunch fescue (*Festuca idahoensis* Elmer) and bluebunch wheatgrass (*Agropyron spicatum* (Pursh) Scribn. and Smith), which are widespread on Washington ranges (identified by Hitchcock (8)³); (2) the digestibility of a mixture of mature bluebunch wheatgrass and other forages occurring on a typical range; (3) the nutritive value of the top half of the bluebunch wheatgrass plant as compared to that of the whole plant cut about 1 inch above the crown of the bunch, in range mixtures composed largely of this grass; (4) the supplementary effect of ground Beldi barley and old-process linseed cake when fed with range mixtures composed largely of mature bluebunch wheatgrass; (5) the supplementary effect of linseed cake when fed at two levels; and (6) the performance of lambs and ewes fed similarly in metabolism experiments.

REVIEW OF LITERATURE

DIGESTION STUDIES

Kennedy and Dinsmore (10) reported one of the earliest studies on digestibility of range feeds. They fed green range forages cut in early summer to 3-year-old wethers for a period of 6 days. The green grasses were digested fairly well, but in most cases the other range forages were more completely digested.

Dinsmore and Kennedy (4) fed several native hays composed of a mixture of grasses and clover, but with a predominance of one grass, to 3-year-old wethers during a 5-day experimental period. All the nutrients were quite completely digested, and these hays were considered excellent fattening feeds.

Hart and his coworkers (6) studied the digestibility of range grass composed largely of soft chess (*Bromus hordeaceus* L.) gathered in September after the seed heads had shattered. They recorded a negative value for crude-protein digestibility. The wether lambs to which this grass was fed lost 3 to 5 pounds' weight in a 10-day feeding period, and their maximum consumption was a little under 1 pound daily, indicating the unpalatability of the grass. Watson and Horton

¹ Received for publication October 17, 1939. Published as Scientific Paper No. 389, College of Agriculture and Experiment Station, State College of Washington.

² The author wishes to express his appreciation for helpful suggestions and criticisms to J. Sotola and H. Hackedorn of the Washington Agricultural Experiment Station and to E. H. Steffen of the College of Agriculture.

³ Italic numbers in parentheses refer to Literature Cited, p. 49.

(20) evaluated grass-hay quality on the basis of its digestible crude-protein content.

Knight, Hepner, and Nelson (11) concluded that the reason forages cure so well on the range and retain their nutritive value to a great extent is their high crude-fiber content. However, Hopper and Nesbitt (9) observed that a high crude-fiber content in range grasses and hays depressed their digestibility.

Christensen and Hopper (3) made a seasonal study of the digestibility of prairie hay fed to steers. These hays consisted of from 50 to 75 percent of western needlegrass or needle-and-thread grass (*Stipa comata* Trin. and Rupr.) and 25 to 50 percent of weeds and other grasses. The July cutting taken at the height of the growing season was most palatable, highest in nutrient content, and most digestible. The annual and biennial cuttings were quite similar in composition and digestibility. The April and October cuttings were similar in composition, but their digestibility was lower because of the old grass contained in the hay.

Armsby (1) concluded that there are comparatively small differences in the digestibility of identical feeds when consumed by the various species of ruminants unless the crude-fiber content is high, in which case cattle are superior to sheep in their ability to digest such feeds. Forbes and his coworkers (5) found that sheep digested all nutrients except crude fiber more efficiently than cattle in a ration containing good-quality roughage and concentrates, but cattle excelled in ability to digest crude fiber.

In digestion experiments carried on by Roberts (15), a native hay composed largely of western wheatgrass or bluestem (*Agropyron smithii* Rydb.) proved to be a more valuable feed than timothy hay.

Headden (7) reported that the native hay of Colorado was considerably superior to timothy in its digestible crude-protein content, but contained about the same amount of digestible dry matter. McCreary (12) concluded that after range grasses mature in the fall, they have a feeding value that appears to be little better than that of oat straw.

CONCENTRATE SUPPLEMENTS TO BUNCH GRASS

Mature range grasses and hays have wide nutritive ratios, according to Hopper and Nesbitt (9), and require a high-protein feed to supplement them so that sufficient protein will be available to meet the requirements of production, reproduction, and growth.

Anderson⁴ contends that a winter supplement is necessary for ewes wintered on mature bunch grass unless they go into the winter in high condition, for without it many are unable to withstand the severe storms that often occur in the Northwest. He observed that cottonseed cake was superior to corn or beet-pulp pellets as a supplement to a bluebunch fescue range near Bozeman, Mont. However, Smith (17) found little difference between corn and cottonseed cake as supplements to the short-grass range near Miles City, Mont. This was probably due to the higher crude-protein content of the grass at Miles City. Maynard (13) reported an experiment in Utah where one-third of a pound of cottonseed cake was fed daily with wild hay, which is low in protein, to old range ewes. Lambs were produced

⁴ ANDERSON, I. M. C. WINTERING EWES ON THE RANGE. U. S. Dept. Agr., Ext. Anim. Husbandman Ser. 40: 12-15. 1935. [Micrographed.] See p. 15.

that weighed 6 pounds per head more by June 1 than lambs from ewes fed one-third of a pound of shelled corn with the same kind and amount of hay.

CHARACTERISTICS OF GRASSES FED

The Forest Service (19, pp. G 6) reported that bluebunch fescue is quite palatable in the spring when it is young and tender and again in the fall when cured, but in the summer when it becomes rather tough and dry, it is not so readily grazed.

Observations of the Forest Service (19, pp. G 58) indicated that bluebunch wheatgrass is palatable both green and cured, and that each plant has a large amount of tender foliage. It is found on early and late ranges where other palatable grasses are apt to be scarce, but, according to Sampson (16), it does not produce a heavy stand. McCreary (12) analyzed several range grasses and found bluebunch wheatgrass to be lowest in crude protein and comparatively high in ash and crude fiber.

EXPERIMENTAL ANIMALS, MATERIALS, AND METHODS

Range lambs of mixed breeding were used as experimental animals in 1930 and 1933, and in 1932 purebred Rambouillet lambs and pregnant Rambouillet ewes were fed. A detailed report of the method of experimentation, as well as of the metabolism crates used, has been previously presented by Sotola (18) of this station.

All sheep used in this study were given all the forage they would consume without waste. There was a little refuse feed in a few cases, but this was analyzed and deducted. A prescribed amount of concentrate was fed.

The pure stands of bunch grass were gathered on a south slope near Pullman, Wash. on a silt loam type of soil. The range mixtures were collected on a typical range of the semiarid hills of central Washington near Prosser on a sandy loam to a silt loam soil. The range mixtures gathered in 1930, 1932, and 1933 consisted of 90, 93, and 97 percent, respectively, of bluebunch wheatgrass. In 1930 the remainder consisted of about equal parts of the tender tips of common sagebrush *Artemisia tridentata* Nutt.), Russian-thistle *Salsola pestifer* A. Nels.), and Jim Hill mustard *Sisymbrium altissimum* L.). In 1932, 5 percent of sage tips and 1 percent of Russian-thistle and Jim Hill mustard were added to the bunch grass. In 1933 only 3 percent of sage tips were added as there was little other range forage available that was palatable to sheep, and the sage tips were not as luxuriant as in other years.

All the grasses and other forages studied were collected from late October to the first part of December. The seeds of these grasses were almost completely shattered out when collected.

The grass in the range mixture collected in 1930 consisted of the whole plant cut about 1 inch above the crown of the bunch with a hand scythe. As the digestibility of this mixture was very low, only the top half of the grass plant was collected in 1932 and 1933 to see if this portion would prove more digestible. This method of cutting more nearly simulates the grazing of sheep having access to sufficient range.

Coarsely ground Beldi barley (*Hordeum vulgare* L.) and pea-size old-process linseed cake were fed as supplements to the range mixtures. The concentrates were about average in quality, and were fed mixed with the chopped forage.

Analytical methods as reported by the Association of Official Agricultural Chemists (2) were used.

CHEMICAL COMPOSITION OF FEEDS

The chemical analyses of the grasses, range mixtures, concentrates, and rations, including range mixtures and concentrates, are summarized in table 1. The grasses and range mixtures were quite high in crude fiber and low in crude protein. These tendencies were not so great in bluebunch fescue.

Bluebunch fescue contained more crude protein and ash and less crude fiber than bluebunch wheatgrass. The range mixture collected in 1933 contained less ash and crude fat and more crude fiber than that collected in 1932. In both of these collections, only the top half of the bunch was used. Most of the growing season of March, April, May, and June of 1933 was cooler and the precipitation greater than in the similar period of 1932. This seasonal variation in nutrient content is in accord with the results of Woodman and his co-workers (21) in their study of pasture grasses. The range mixture collected in 1932 was particularly high in crude fat, and the grass included in the range mixture had the golden color that stockmen associate with high feeding value.

TABLE 1.—Chemical composition of range grasses, range mixtures, concentrates, and mixed rations

Feed	Year forage collected	Part of plant collected	Ex- peri- ment No.	Dry matter	Ash	Crude protein (N×6.25)	Carbohydrates		Crude fat
							Crude fiber	Nitro- gen-free extract	
				Percent	Percent	Percent	Percent	Percent	Percent
Bluebunch fescue	1930	Whole plant.	2	87.70	14.60	4.62	27.18	38.65	2.65
Bluebunch wheat- grass.	1930	do	1	88.74	10.14	2.94	33.37	39.61	2.68
Range mixture	1930	do	3	88.06	7.96	3.73	29.36	43.54	3.47
Do	1932	Top half	4	89.91	12.88	2.49	31.52	38.29	4.73
Do	1932	do	5, 6	90.70	12.82	2.44	31.80	38.87	4.77
Do	1932	do	7	91.17	12.89	2.45	31.74	39.29	4.80
Do	1932	do	9, 10	91.29	12.91	2.45	32.00	39.13	4.80
Do	1933	do	11	86.99	6.62	2.12	34.50	41.87	1.88
Do	1933	do	12	88.12	6.84	2.09	34.75	42.52	1.92
Do	1933	do	13	89.09	6.93	2.06	36.87	41.79	1.44
Do	1933	do	14	88.17	6.51	2.43	36.89	40.94	1.40
Barley			5	90.30	2.84	5.59	5.00	72.02	1.55
Do			7	89.29	2.57	10.34	5.54	69.31	1.53
Do			12	87.44	2.88	9.66	4.70	68.70	1.50
Linseed cake			6	92.40	7.35	32.23	7.81	39.46	5.55
Do			9, 10	92.27	8.29	35.00	7.80	35.04	5.54
Do			13	89.80	5.81	33.73	8.12	36.75	5.39
Do			14	89.50	5.79	34.44	8.21	35.68	5.38
Range mixture plus—									
Barley	1932		5	88.49	8.45	4.92	20.39	51.51	3.22
Linseed cake	1932		6	91.20	10.97	12.48	23.76	38.96	5.03
Barley	1932		7	90.05	8.44	5.82	21.81	50.73	3.25
Linseed cake	1932		9	91.34	12.20	7.50	28.85	38.80	4.99
Do	1932		10	91.61	11.38	13.42	23.99	37.78	5.04
Barley	1933		12	87.86	5.35	4.94	23.43	52.38	1.76
Linseed cake	1933		13	89.18	6.80	5.86	38.42	41.19	1.91
Do	1933		14	88.49	6.34	10.06	30.05	39.69	2.35

RESULTS OF THE DIGESTION STUDIES

FEED CONSUMPTION

Table 2 shows the amount of nutrients consumed by the lambs and ewes. Because of the difference in size of the experimental sheep in the 3 years of this study, the feed consumption was computed to the basis of 100 pounds of body weight. The lambs fed range mixture and the high level of linseed cake in experiment 14 consumed the largest amount of dry matter, crude protein, crude fiber, and nitrogen-free extract. The addition of linseed cake to the range mixtures fed in 1932 and 1933 considerably increased the palatability of these forages. The addition of barley did not have as much effect in this respect. The second largest amount of dry matter consumed was from the range mixture collected in 1930, and more forage dry matter was consumed than in any other ration. The lowest dry-matter consumption by lambs was from the range mixture collected in 1933, which was quite high in crude fiber and low in crude fat. Although there was a slight difference in forages other than grass in the range mixture collected in 1930 and 1933, and the growing and maturing seasons were somewhat different, the considerable variation in the consumption of these forages would indicate that the top half of the

TABLE 2.—Relative amount of feed and nutrients consumed daily by lambs and ewes fed bunch grasses and range mixtures alone and with concentrates

[Computed to 100 pounds (45.36 kg.) body weight]

Feed, level, and year forage collected	Experiment No.	Sheep	Kind of sheep	Average weight of sheep		Kind of feed		Feed and nutrients consumed per head daily, computed to 100 pounds body weight						
				Forage	Concentrate	Forage	Concentrate	Feed as fed	Dry matter	Ash	Crude protein (N X 6.25)	Crude fi- ber	Nitrogen- free ex- tract	Crude fat
				Kg.	Pct.	Pct.		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
Bluebunch fescue— pure stand:		No.	Lambs											
1930 ¹	2	6	Lambs	24.7	100.00	0.00		901.5	790.6	131.6	41.6	245.0	348.5	23.9
Bluebunch wheatgrass— pure stand:			do											
1930 ¹	1	6	do	24.2	100.00	.00		780.2	692.5	79.1	22.9	290.4	309.2	20.9
Range mixture:			do											
1930 ¹	3	6	do	24.4	100.00	.00		1,005.8	885.7	80.1	37.5	295.3	437.9	34.9
1933 ²	11	6	do	29.6	100.00	.00		331.5	288.4	21.9	7.0	114.4	138.9	6.2
1932 ³	4	6	Ewes	65.0	100.00	.00		323.5	290.8	41.7	8.0	102.0	123.8	15.3
Range mixture plus barley—high level:			Lambs											
1932.....	7	3	Lambs	45.7	55.87	44.13		589.5	530.8	49.8	34.3	128.6	298.9	19.2
1933.....	12	6	do	29.2	62.33	37.67		724.5	636.5	38.8	35.8	169.7	379.4	12.8
1932.....	5	3	Ewes	69.1	55.50	44.50		486.6	430.6	41.1	23.9	99.2	250.7	15.7
Range mixture plus lin- seed cake—low level:			Lambs											
1932.....	9	3	Lambs	45.7	85.29	14.71		607.2	554.6	74.1	45.5	172.1	232.6	30.3
1933.....	13	6	do	28.3	87.98	12.02		801.4	714.7	54.5	47.0	267.8	330.1	15.3
Range mixture plus lin- seed cake—high level:			do											
1932.....	10	3	do	51.2	66.90	33.10		744.9	682.4	84.8	100.0	178.7	281.4	37.5
1933.....	14	6	do	29.9	76.16	23.84		1,118.7	989.9	70.9	112.5	336.2	444.0	26.3
1932.....	6	3	Ewes	62.2	66.21	33.79		713.7	650.9	78.3	89.1	189.6	278.0	35.9

¹ Grass cut about 1 inch above the crown of the bunch.

² Bluebunch wheatgrass in range mixture cut about halfway from crown to top of bunch.

³ 2 years old.

bluebunch wheatgrass plant was less palatable than the whole plant. When no concentrate was fed, the consumption of bunchgrass and range mixtures was inadequate for maintenance except in experiment 3 with the range mixture collected in 1930. But according to Armsby (1) the amount of feed consumed has little effect on its digestibility as long as the kind of feed consumed remains the same and the experimental animals are similar.

COEFFICIENTS OF APPARENT DIGESTIBILITY

The average coefficients of apparent digestibility obtained with lambs and ewes for the organic nutrients of the rations fed are summarized in table 3. Six lambs were used except in 1932, when three lambs were fed one ration and the other three another ration as indicated in table 3.

TABLE 3.—*Coefficients of digestibility of bunch grasses and range mixtures fed alone and supplemented with concentrates to lambs and ewes*

[Standard deviations ¹ are reported only where six sheep were fed]

Feed, level, and year forage was collected	Experiment No.	Sheep	Kind of sheep	Coefficients of digestibility				
				Dry matter	Crude protein (N×6.25)	Carbohydrates		Crude fat
						Crude fiber	Nitrogen-free extract	
		Number		Percent	Percent	Percent	Percent	Percent
Bluebunch fescue—pure stand:								
1930 ²	2	6	Lambs	41.5±2.08	21.2±5.00	55.3±7.12	49.8±2.23	33.5±2.26
Bluebunch wheatgrass—pure stand:								
1930 ²	1	6	do	36.0±2.76		50.7±2.66	38.8±3.06	32.0±2.00
Range mixture:								
1930 ²	3	6	do	47.5±2.26	17.2±6.43	55.7±2.66	49.8±2.23	44.7±2.50
1933 ³	11	6	do	51.0±8.03		67.0±8.54	52.6±8.79	49.4±4.16
1932 ⁴	4	6	Ewes	27.8±5.31		43.5±4.68	33.5±5.79	27.2±6.85
Range mixture plus barley—high level:								
1932	7	3	Lambs	58.7	47.0	57.3	71.0	47.0
1933	12	6	do	59.2±3.31	34.4±6.19	57.3±6.06	68.2±2.64	58.7±4.72
1932	5	3	Ewes	47.3	33.0	37.0	60.3	45.3
Range mixture plus linseed cake—low level:								
1932	9	3	Lambs	40.3	37.0	57.0	41.0	69.7
1933	13	6	do	52.3±4.32	38.0±7.35	65.5±5.24	51.5±3.98	58.7±5.13
Range mixture plus linseed cake—high level:								
1932	10	3	do	53.0	67.7	56.3	58.3	66.7
1933	14	6	do	56.0±2.76	62.3±3.39	67.0±3.41	54.8±2.48	65.8±4.17
1932	6	3	Ewes	45.3	63.3	53.0	52.3	52.0

¹ The formula used in computing the standard deviation was $\sigma = \sqrt{\frac{\sum d^2}{(n-1)}}$

² Grass cut about 1 inch above the crown of the bunch.

³ Grass in range mixture cut about halfway from crown to top of bunch.

⁴ 2 years old.

All the nutrients of the range mixture fed in 1930, which consisted largely of bluebunch wheatgrass, were more completely digested than those in the pure stand of this grass. The digestibility of the dry matter of the mature grasses and the range mixtures was quite low, with the exception of the range mixture collected in 1933. The consumption of the 1933 range mixture was low, but, according to Armsby (1), this should make little difference in its digestibility. The addition of barley and linseed cake to the range mixture increased the

digestibility of dry matter of the mixed ration in all cases as compared to that of the range mixture fed alone.

More crude protein was excreted than was consumed by lambs fed the pure stand of bluebunch wheatgrass and the 1933 range mixture, and by ewes fed the 1932 range mixture. According to Mitchell (14) the greater the concentration of indigestible, nonnitrogenous material in a ration, the greater the fecal excretion of metabolic nitrogen. The addition to range mixtures of barley and of linseed cake (particularly the larger amount) materially increased the digestibility of crude protein and crude fat. No similar increase was noted in the digestibility of crude fiber, and only when barley was added to the range mixture was there a significant increase in the digestibility of nitrogen-free extract.

The coefficients of digestibility obtained with lambs were in all cases higher than with ewes similarly fed. The ewes fed in these experiments were well along in their pregnancy period.

The range mixtures fed with linseed cake at the low level were in general less completely digested than when fed with barley at the high level, which was about three times as much concentrate. However, only the dry matter and nitrogen-free extract of the latter ration were more completely digested than that of the range mixtures and the larger amount of linseed cake. About one-half pound of concentrate per 100 pounds of live weight was fed at the high level.

TOTAL DIGESTIBLE NUTRIENT RATING

Table 4 presents a summary of the digestible nutrients in the rations fed and a rating of the rations based on the total digestible nutrients contained.

TABLE 4.—*The digestible nutrients in bunch grasses and range mixtures fed alone and with concentrates; rating based on total digestible nutrients*

Feed level, and year forage was collected	Ex- peri- ment No.	Kind of sheep	Total dry matter in ration	Digestible nutrients					Nutri- tive ratio, 1 to —	Rating on basis of total dige- stible nu- trient
				Crude pro- tein (N X 6.25)	Carbo- hydrates		Crude fat	Total		
					Crude fiber	Nitro- gen- free extract				
Bluebunch fescue— pure stand: 1930.....	2	Lambs	Per- cent 87.70	Per- cent 0.98	Per- cent 15.03	Per- cent 19.32	Per- cent 0.89	Per- cent 37.33	37.09	Per- cent 88.67
Bluebunch wheatgrass —pure stand: 1930.....	1	do	88.74		16.92	15.37	.86	34.23		81.31
Range mixture: 1930.....	3	do	88.06	.64	16.29	21.68	1.55	42.10	64.78	100.00
1933.....	11	do	86.99		23.12	22.02	.93	47.23		112.18
1932.....	4	Ewes ¹	89.91		13.71	12.83	1.29	29.44		69.93
Range mixture plus barley—high level: 1932.....	7	Lambs	90.05	2.74	12.50	36.02	1.53	54.70	18.96	129.93
1933.....	12	do	87.86	1.69	13.42	35.72	1.03	53.15	30.45	126.25
1932.....	5	Ewes ¹	88.49	1.62	7.54	31.06	1.46	43.50	25.85	103.32
Range mixture plus lin- seed cake—low level: 1932.....	9	Lambs	91.34	2.78	16.16	15.70	3.48	42.48	14.28	100.90
1933.....	13	do	89.18	2.23	21.89	21.21	1.12	47.85	20.46	113.66
Range mixture plus lin- seed cake—high level: 1932.....	10	do	91.61	9.08	13.51	22.02	3.36	52.17	4.74	123.92
1933.....	14	do	88.49	6.27	20.13	21.75	1.55	51.64	7.24	122.66
1932.....	6	Ewes ¹	91.20	7.90	12.59	20.38	2.62	46.77	4.92	111.09

¹ 2 years old.

When the total digestible nutrients of the range mixture fed in 1930 were figured at 100 percent, the range mixture and barley combinations fed to lambs in 1932 and 1933 were highest, with ratings of 129.93 percent and 126.25 percent, respectively. The range mixture fed to ewes in 1932 rated only about one-half as high, or 69.93 percent. These ewes consumed a small amount of forage and its digestibility was quite low. All rations containing some concentrate rated 100 percent or higher.

The nutritive ratios of the grasses and of range mixtures fed alone were quite wide in cases where there were positive coefficients of digestibility of crude protein. The nutritive ratios were very narrow only when the high level of linseed cake was fed with the range mixtures.

NITROGEN BALANCES

The nitrogen consumption and retention for lambs and ewes during the 10-day period on the different feeds are reported in table 5. The addition of the larger amount of linseed cake to the range mixtures more than doubled the nitrogen intake over that from range mixtures and barley. The smallest nitrogen consumption from the rations containing no concentrate was 7.28 gm. as compared with 118.16 gm., the smallest amount consumed when the linseed cake was fed at the high level. More than 16 times as much nitrogen was consumed in the latter ration. The nitrogen consumption in the former case was very low because of the small amount of feed eaten and its low nitrogen content.

TABLE 5.—Summary of nitrogen balances for lambs and ewes fed bunch grasses and range mixtures alone and supplemented with concentrates; 10-day period

[Standard deviations given only for experiments with 6 sheep]

Feed, level, and year forage was collected	Experi- ment no.	Sheep Number	Kind of sheep	Nitrogen consumed ¹	Nitrogen voided in—			Nitrogen balance	Intake stored
					Feces	Urine	Total		
Bluebunch fescue—pure stand:									
1930 1.....	2	6	Lambs	36.37±5.50	28.40±3.98	15.54±1.97	44.02±4.87	Grams -7.65	Percent
Bluebunch wheatgrass—pure stand:									
1930 1.....	1	6	do	19.50±3.68	22.72±3.42	13.72±1.81	36.44±3.76	-16.94	
Range mixture:									
1930 1.....	3	6	do	32.38±4.55	26.73±4.50	13.56±2.43	40.31±6.12	-7.93	
1933 1.....	11	6	do	7.28±2.63	12.57±4.15	20.90±2.65	33.47±3.00	-26.19	
1932 2.....	4	6	Ewes ²	18.60±5.30	28.20±6.46	43.46±7.96	71.66±10.00	-53.06	
Range mixture plus barley—high level:									
1932.....	7	3	Lambs	55.39	30.53	19.26	48.81	+6.78	12.20
1933.....	12	6	do	36.73±1.69	24.14±2.29	15.33±5.78	39.47±2.70	-2.74	
1932.....	5	3	Ewes ²	59.13	40.01	28.93	68.94	-9.81	
Range mixture plus linseed cake—low level:									
1932.....	9	3	Lambs	70.68	44.30	42.00	86.30	-15.71	
1933.....	13	6	do	46.87±1.71	29.04±4.36	23.53±2.20	52.57±5.69	-5.70	
Range mixture plus linseed cake—high level:									
1932.....	10	3	do	178.43	58.11	85.94	144.05	+34.38	19.27
1933.....	14	6	do	118.10±2.65	44.47±4.63	43.41±6.60	87.86±5.86	+30.28	25.60±5.27
1932.....	6	3	Ewes ²	195.01	71.70	123.63	195.33	-32	

¹ Grass cut about 1 inch above crown of bunch.² Bluebunch wheatgrass in range mixture cut about halfway from crown to top of bunch.³ 2 years old.

There was no nitrogen retention from bunch grasses or range mixtures fed with no concentrate supplement, and in only one case when barley was fed with a range mixture. However, all the lambs fed the higher level of linseed cake stored about one-fifth to one-fourth of the nitrogen consumed, and the nitrogen consumed and voided by the ewes on this feed just about balanced. Linseed cake was decidedly superior to barley as a supplement from the standpoint of nitrogen retention.

SUMMARY AND CONCLUSIONS

Bluebunch fescue (*Festuca idahoensis*, Elmer) cut in the late fall of 1930 about 1 inch above the crown of the bunch contained more crude protein, less crude fiber, and more total digestible nutrients when fed to lambs than bluebunch wheatgrass (*Agropyron spicatum* (Pursh) Scribn. and Smith). Coefficients of digestibility of crude protein were positive for bluebunch fescue and negative for bluebunch wheatgrass, although lambs fed both of these grasses registered negative nitrogen balances. The bluebunch fescue was more palatable than the bluebunch wheatgrass.

A range mixture consisting of bluebunch wheatgrass with 10 percent of other forages collected in the late fall of 1930 contained more crude protein, nitrogen, free extract, and crude fat, less crude fiber and ash, and was more digestible and palatable than the same grass fed alone. The bluebunch wheatgrass of this mixture was also cut about 1 inch above the crown of the bunch. The range mixture of 1930 contained less crude fiber, more crude fat, more crude protein of higher digestibility, and was considerably more palatable than the range mixture collected in 1933, which contained only the top half of the bluebunch wheatgrass. The range mixture collected in 1932, similar in physical composition to that of 1933, but containing considerably more crude fat and ash, a little less crude fiber and nitrogen-free extract, and about the same amount of total digestible nutrients, seemed to be more palatable than that collected in 1933 when fed in combination with ground Beldi barley and old-process linseed cake. These comparisons would indicate that the larger proportion of basal leaves in the 1930 range mixture more than compensated for the lower part of the stems included as compared to the 1932 and 1933 range mixture. The range mixture collected in 1930 furnished enough digestible nutrients to meet the maintenance requirements of the lambs to which it was fed, but the other range mixtures, as well as the pure stands of bluebunch fescue and bluebunch wheatgrass, failed to meet these requirements.

When approximately one-half pound of linseed cake per 100 pounds of live weight was fed with range mixtures to lambs and pregnant ewes, the mixed rations were more palatable and digestible than the same range mixtures fed alone. This combination was also more palatable and contained more digestible crude protein, crude fiber, and crude fat than a similar amount of ground barley fed with the same range mixture, but the barley combination contained slightly more total digestible nutrients because of a greater content and digestibility of nitrogen-free extract.

A ration consisting of approximately one-sixth of a pound of linseed cake per 100 pounds live weight of lambs and a range mixture collected in 1933, was more palatable and contained more total digestible

nutrients than a similar range mixture fed alone. This ration was also more palatable than approximately one-half pound of barley and a similar range mixture, but did not contain as much total digestible nutrients as the latter ration. Approximately one-half pound of linseed cake and range mixture was more palatable and digestible than the smaller amount of linseed cake and range mixture, and the former ration was the only one of this study that had a very narrow nutritive ratio.

Pregnant ewes were less efficient than lambs in digesting range mixtures with or without the addition of barley. They more closely approached lambs in efficiency of digestion when fed linseed cake as a supplement to a similar range mixture.

It would seem from this study that mature bluebunch fescue and bluebunch wheatgrass collected in late fall have been overrated as feed for sheep.

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RELATION BETWEEN CAROTENOID CONTENT AND NUMBER OF GENES PER CELL IN DIPLOID AND TETRAPLOID CORN¹

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INTRODUCTION

The evaluation of induced chromosome doubling as a method of plant breeding requires more information on the specific effects of chromosome doubling than is available at the present time. It is important that this information be procured from comparisons of related diploid and tetraploid strains of known origin and similar genetic constitution. Such strains have not been generally available because of the infrequency with which chromosome doubling occurs spontaneously and because of the lack of methods for the experimental production of autotetraploids generally applicable to crop plants. But with the development of the heat-treatment technique for the experimental doubling of chromosome numbers (10)³ comparable diploid and tetraploid strains of corn (*Zea mays* L.) and other plants have been made available for study.

The effect of chromosome doubling on the carotenoid pigments in corn is of special interest because of the vitamin A potency of two of these pigments. The investigation reported in this paper was undertaken to determine the relation between carotenoid content, cell volume, and gene number in comparable strains of diploid and tetraploid corn and to determine the carotenoid content of examples of commercial varieties, inbred strains, and hybrids of ordinary diploid corn. A preliminary report of these studies has already been published (12).

The carotenoids of the corn kernel are located in the endosperm tissue, which is relatively homogeneous in cellular organization and is thus favorable material for a study of cell-volume relations. In corn of the ordinary diploid sort the chromosomes and genes are present in triplicate, while in the derived tetraploid there are six sets of chromosomes and genes. Since the character in question is definitely localized in the endosperm tissue, a favorable opportunity is presented for a study of the effect of gene number on the degree of development of the yellow pigment.

Yellow corn meal contains the carotenoid pigments beta-carotene and cryptoxanthin, precursors of vitamin A, and zeaxanthin, which

¹ Received for publication September 25, 1939. Cooperative investigation of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Departments of Botany and Dairy Industry, New York State College of Agriculture, aided by a grant from the committee on radiations of the National Research Council.

² The carotenoid analyses were made by R. G. Hart in the dairy chemistry laboratory, New York State College of Agriculture.

³ Italic numbers in parentheses refer to Literature Cited, p. 64.

has no relation to vitamin A activity (7). In animal experiments, Steenbock and Boutwell (14) demonstrated the vitamin A potency of yellow corn and the lack of potency of white corn. Later, Mangelsdorf and Fraps (8) and Johnson and Miller (3, 4) found a direct quantitative relationship between the amount of vitamin A potency and the number of dominant genes for yellow in comparable samples of corn.

Studies on the chemical composition of diploids and their autotetraploid derivatives have been made in relatively few plants. The relation between chromosome number and vitamin C content in apples was studied by Crane and Zilva (1), who found that triploid varieties had more of the vitamin than did the unrelated diploid varieties that were examined. Autotetraploid tomatoes also contain more vitamin C than the parental diploids, according to Sansome and Zilva (13). Chemical analyses of comparable diploid and autotetraploid strains of tomatoes and petunias were made by Kostoff and Axamitnaja (5), who reported that the tetraploid tomatoes had more nitrogen and water but less cellulose and ash than the parental diploid strain; but in *Petunia* the chemical composition of the diploid and tetraploid was essentially the same. The results with tomatoes should be interpreted in the light of the fact that tetraploid tomatoes, because of their reduced fertility, have smaller fruits than the diploids.

PREPARATION OF MATERIALS

The strains of diploid and tetraploid yellow corn selected for comparison were derived from three inbred lines of pure yellow corn, Webber Dent 2312, Illinois A-2311, and Luces Favorite W36-1. The Webber Dent inbred was crossed with Illinois A, and the F_1 hybrid was crossed in turn with the Luces Favorite inbred. Chromosome doubling was induced in this three-way hybrid by the heat-treatment technique (10), and the resulting tetraploid plants, several in number, were mass-pollinated for two generations to provide adequate material for analysis. A comparable diploid strain was developed from the same source by mass-pollinating for two generations the diploid sister plants of the induced tetraploid individuals. The two strains were designated Diploid Yellow and Tetraploid Yellow, respectively. A second tetraploid yellow strain, designated Tetraploid Yellow B, was also analyzed for total carotenoid content. This strain originated from a cross between an inbred white flint corn and the Webber Dent 2312 inbred, followed by three generations of selective breeding for the yellow character. Comparable diploid and tetraploid strains of white corn, derived from inbred lines of white Argentine Flint and Spanish Flint and here designated Diploid White and Tetraploid White, were also analyzed for carotenoid content. In addition, separate analyses were made of a number of diploid inbred lines, including those from which the above-mentioned strains were derived.

In addition to these analyses of tetraploid and diploid corn, the carotenoids were determined in examples of various commercial varieties, inbred lines, and their hybrids, including a commercial double-cross hybrid grown extensively for grain and fodder in New York.⁴

⁴ The hybrid, W29-3, and the parent lines, W36-1, W36-2, W36-3, and W36-4, were produced by Dr. R. G. Wiggans, of the Department of Plant Breeding, New York (Cornell) Agricultural Experiment Station, and were furnished by him for these studies.

The comparison of cell-volume relations in the endosperm tissue of the diploid and tetraploid corn was based upon a study of morphologically mature kernels selected from the pure yellow strains, which were analyzed for carotenoid content. Microtome sections were prepared in the usual manner from the desired portions of kernels that had been fixed in a weak Flemming's solution. To facilitate the preparation of the sections, the reserve starch in the endosperm was partly removed by soaking the seeds in water prior to fixation. Cell volumes were computed from measurements taken from camera-lucida sketches drawn at a magnification of 440 diameters.

ANALYTICAL METHOD

For the fractionation and determination of the carotenoids in the corn meal, the following simplified method, based upon the procedure of Kuhn and Brockmann (6), was developed. The meal was prepared from entire kernels, including the embryo and pericarp in addition to the endosperm. Since the endosperm comprised the bulk of the meal and the proportions of the different parts were very similar in the diploid and tetraploid stocks that were analyzed, the presence of the germ and pericarp was disregarded in comparing the relative amounts of carotenoid present in the samples. Samples were prepared for analysis by selecting at random 10 ears of corn from each strain. Corn meal was produced from the air-dried, shelled grain by grinding 50-gm. samples for 5 minutes in a Wiley mill equipped with a sieve of 1-mm. mesh. The meal was dried in a desiccator over phosphorus pentoxide (P_2O_5). Four to eight samples of 2.5 gm. each were thoroughly mixed with 50 ml. of anhydrous methyl alcohol (distilled from lime) and allowed to stand in glass-stoppered flasks for 18 hours. The methyl alcohol extract was filtered through sintered glass, the filter and precipitate were washed with small portions of methyl alcohol, and the volume was reduced by evaporation under reduced pressure and then made up to exactly 50 ml. with additional methyl alcohol. The total pigment in the methyl alcohol was determined in a photoelectric colorimeter with Corning glass filters 585 and 428. The construction, calibration, and use of this colorimeter has been described by Hand and Sharp (1a). The extract was saponified for 2 hours at 50° C. after 5 ml. of a 5-percent solution of potassium hydroxide (KOH) in methyl alcohol had been added. The solution was cooled, 6.0 ml. of water added, and the mixture shaken for 15 seconds in a separatory funnel. The active fraction, containing beta-carotene and cryptoxanthin, was extracted with from 4 to 8 successive 25-ml. portions of petroleum ether.⁵ The petroleum ether extracts were combined and evaporated to exactly 50 ml. under reduced pressure, and the absorption coefficient was determined in the colorimeter. In a similar manner the inactive zeaxanthin was extracted with petroleum ether after the water content of the methyl alcohol had been increased to 40 percent, and the absorption coefficient in petroleum ether was determined.

Calculations of carotenoid were made from a calibration curve for the absorption coefficient of pure beta-carotene plotted against

⁵ Separate determination of beta-carotene and cryptoxanthin can be made by extracting the beta-carotene from 99-percent methyl alcohol and the cryptoxanthin from 90-percent methyl alcohol, but were not made in these studies because of the relatively small amount of beta-carotene in corn meal.

milligrams of beta-carotene per liter of petroleum ether (fig. 1). In these calculations, it was assumed that cryptoxanthin and zeaxanthin had the same absorption as beta-carotene with glass filters 585 and 428 and that the light absorption was the same in methyl alcohol as in petroleum ether. Since these assumptions are not exactly correct, an error was introduced with respect to the absolute amounts of carotenoid, but not with respect to the relative values for the different

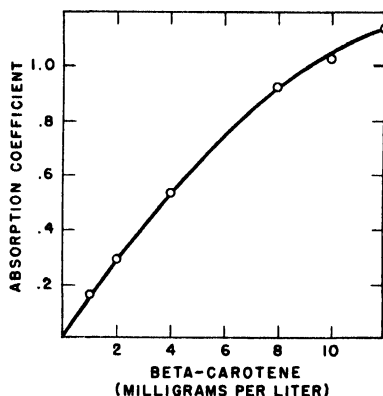


FIGURE 1.—Light absorption by beta-carotene in petroleum ether. Absorption coefficient, $\log_{10} \frac{I_0}{I}$ for 1 cm., filters 585 and 428.

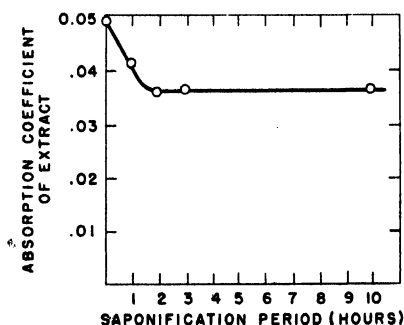


FIGURE 3.—Effect of saponification time on the extraction of carotenoids by petroleum ether from 85-percent methanol.

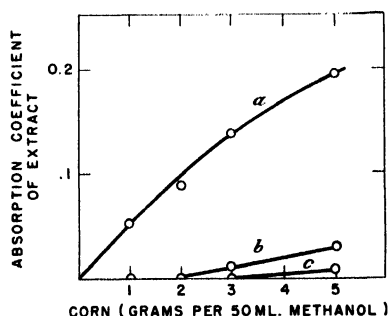


FIGURE 2.—Repeated extractions of corn samples by methanol: *a*, First extraction; *b*, second extraction; *c*, third extraction.

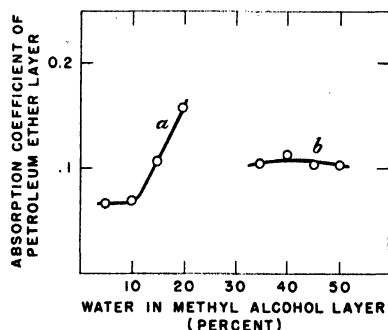


FIGURE 4.—Effect of water content on the fractionation of carotenoids: *a*, Active fraction; *b*, inactive fraction.

strains of corn. In practice the calculation was further simplified so that the number of milligrams of carotenoid per gram sample was obtained by multiplying the absorption coefficient by the factor 0.155. This approximate method neglects the deviation from linearity up to 5 mg. per liter (fig. 1). The chief advantage in using the factor is that the calculated values for carotenoid content can be readily changed to the experimental values for the absorption coefficients of 50-ml. extracts from 2.5-gm. samples of corn.

Experiments made in the elaboration of the method are summarized in the accompanying figures. In figure 2 it can be seen that practically all of the carotenoid is removed by a single extraction with 50 ml. of methyl alcohol if the sample is not larger than 2.5 gm. Figure 3 shows that the saponification of the xanthophyll esters is complete after 2 hours at 50° C. If the saponification is incomplete, the xanthophyll esters are extracted with the beta-carotene and cryptoxanthin and the values for the active fraction are erroneously high. If the saponification is carried on too long, e. g., for 10 hours, extraction of the zeaxanthin fraction by petroleum ether is very much more difficult. Figure 4 shows that a sharp separation of the active and inactive fractions is obtained with 10 percent of water in the methyl alcohol. If more water is used for the first extraction, some of the zeaxanthin is forced into the petroleum ether. The amount of water needed for the optimum extraction of the inactive fraction may vary from 30 to 50 percent.

Some idea of the accuracy of the method can be obtained from a series of 17 analyses of the same strain of corn for which the average deviation from the mean was only ± 4.2 percent. By reducing the number of steps in the procedure the chances for the loss or destruction of carotenoids were reduced. The results reported herein agree with typical values for the carotenoids in a sample of Italian corn reported by Kuhn and Grundmann (?) (beta-carotene, 0.0007 mg.; cryptoxanthin, 0.0046 mg.; and zeaxanthin, 0.0127 mg. per gram) but are considerably higher than those of Johnson and Miller (3, 4).

RESULTS OF CAROTENOID ANALYSES

The results of the carotenoid analyses of diploid and related strains of yellow and white corn; of a number of commercial varieties of ordinary diploid corn, including examples of the more important kernel types, such as dent, flint, pop, and sweet corn; and of a limited number of diploid inbred lines and hybrids are shown in table 1. The total carotenoids in methyl alcohol, the active provitamin A fraction, containing carotene and cryptoxanthin, and the inactive zeaxanthin fraction are listed separately in the table. The sum of the active and inactive petroleum ether fractions does not in all cases equal the values obtained for the total carotenoids in methyl alcohol, presumably because of losses incurred during their saponification, transfer to petroleum ether, and final separation. Therefore, the values obtained from the original methyl alcohol extractions are considered to be a more reliable index of the relative amounts of total carotenoids present in the different kinds of corn that were analyzed.

The feeding experiments of Mangelsdorf and Fraps (8) demonstrated that the amount of vitamin A in corn meal was directly proportional to the number of dominant genes for yellow endosperm color present in the seed, the amount present being approximately in the ratio 3:2:1 for the *YYY*, *YYy*, and *Yyy* endosperm genotypes. White corn of the constitution *yyy* was found to have no vitamin A potency, a result which was in agreement with the earlier work of Steenbock and Boutwell (14) and Hauge and Trost (2). Results similar to those of Mangelsdorf and Fraps (8) were reported recently by Johnson and Miller (3) from spectrophotometric analyses.

TABLE 1.—*Carotenoid analyses of tetraploid and related diploid corn, diploid commercial varieties, inbred lines, and hybrids, in milligrams of pigment per gram of dry corn meal, each value being an average of four or more analyses*

[Standard errors are given for the total carotenoid values]

Corn sample	Total carot- enoids in methyl alcohol	Sum of petro- leum ether fraction	Beta-carot- ene and cryptoxan- thin	Zeaxanthin
	<i>Milligrams</i>	<i>Milligrams</i>	<i>Milligrams</i>	<i>Milligrams</i>
Diploid Yellow.....	0.0261±0.0005	0.0243	0.0097	0.0145
Tetraploid Yellow.....	.0366±.0010	.0314	.0122	.0192
Tetraploid Yellow B.....	.0389±.0006			
Diploid White.....	.0067±.0003	.0049	.0018	.0031
Tetraploid White.....	.0054±.0001	.0042	.0017	.0025
Commercial varieties:				
Leaming Yellow.....	.0266±.0001	.0267	.0068	.0199
Cornell 11.....	.0282±.0002	.0201	.0056	.0145
Alvords White Cap Dent.....	.0073±.0002	.0051	.0019	.0032
Argentine Yellow Flint.....	.0479±.0001	.0422	.0160	.0262
Queen Golden pop.....	.0352±.0002	.0325	.0117	.0208
White Rice pop.....	.0034±.0001	.0027	.0008	.0019
Yankee Cheat flour.....	.0044±.0002	.0028	.0008	.0020
Golden Bantam sweet.....	.0071±.0004	.0126	.0031	.0095
Inbred lines:				
Dutton Flint.....	.0144±.0001	.0113	.0035	.0078
Bloody Butcher.....	.0210±.0002	.0131	.0039	.0092
Illinois A-2311.....	.0257±.0001	.0183	.0047	.0136
Webber Dent 2312.....	.0327±.0001	.0237	.0065	.0172
Lucas Favorite W36-1.....	.0270±.0001	.0271	.0108	.0163
Onondaga White W36-2.....	.0057±.0001	.0038	.0016	.0022
Cornell 11 W36-3.....	.0639±.0006	.0651	.0313	.0338
Bloody Butcher W36-4.....	.0476±.0003	.0421	.0178	.0243
Hybrids:				
W36-2 × W36-1.....	.0109±.0001	.0103	.0043	.0060
W36-3 × W36-4.....	.0705±.0001	.0709	.0328	.0381
W29-3.....	.0326±.0004	.0290	.0119	.0171

In the present study a comparison was made between the carotenoid content of pure yellow diploid corn carrying the three dominant genes, *YYY*, for yellow, and a derived tetraploid with the doubled number of genes, *YYYYYY*, for yellow. Increasing the number of genes for yellow from three to six increased the total carotenoid content from 0.0261 mg. per gram of dry meal in the diploid to 0.0366 mg. in the tetraploid (table 1), an increase of 40 percent. This was the percentage increase for the Tetraploid Yellow strain compared with the related Diploid Yellow strain. There was approximately the same percentage increase for both the active provitamin A fraction containing beta-carotene and cryptoxanthin and the inactive zeaxanthin fraction. That is, the increase in carotenoid pigment carried with it a proportional increase in vitamin A potency. The intensity of endosperm color was approximately the same in the diploid and tetraploid strains.

Although the 40-percent increase in carotenoid content is attributed to chromosome doubling, it is recognized that the growing of the Tetraploid Yellow and Diploid Yellow strains for two generations in order to obtain sufficient material for the carotenoid analyses provided a limited opportunity for the segregation of genes affecting carotenoid content to take place independently within each of these strains. Such genotypic changes might either diminish or accentuate the differences in carotenoid content caused by chromosome doubling. However, the inbred lines from which these strains originated, namely, Illinois A, Webber Dent, and Lucas Favorite, did not differ markedly

in carotenoid content (table 1), and the opportunity for gene segregation to occur was reduced to a minimum by practicing mass pollination rather than selfing or sib-crossing individual plants.

A second tetraploid yellow strain that was analyzed, Tetraploid Yellow B, had a carotenoid content of 0.0389 ± 0.0006 mg. This strain was a third-generation selection for yellow from a cross between Webber Dent 2312 and a white Spanish Flint inbred and was still segregating for endosperm color. The carotenoid content of this impure yellow tetraploid strain was appreciably higher than that of its diploid yellow parent, Webber Dent 2312, which was 0.0327 ± 0.0001 mg. Ordinarily the admixture of white with yellow strains reduces carotenoid content. (See table 1 and the analyses of Mangelsdorf and Fraps (8), Johnson and Miller (3), and others.) The relatively high carotenoid content of the Tetraploid Yellow B strain is attributed to the effect of chromosome doubling.

The diploid inbred lines from which the pure Diploid Yellow and Tetraploid Yellow strains originated, namely, Webber Dent 2312, Illinois A-2311, and Lucas Favorite W36-1, were somewhat different with respect to both endosperm color and carotenoid content. The Webber Dent inbred had the deepest endosperm color and also the highest carotenoid content, but the Lucas Favorite inbred, which had the least endosperm color of the three, had a somewhat higher carotenoid content than the deeper yellow Illinois A line. The mean value for the three inbred lines was 0.0292 mg. per gram of dry meal, as compared with 0.0261 mg. for the diploid strain produced by intercrossing these lines.

The total carotenoid content of the Tetraploid Yellow strain was not twice as great as that of the related Diploid Yellow strain, as might have been expected since other workers have shown that in ordinary diploid corn the carotenoid content is directly proportional to the number of dominant genes for yellow endosperm. The carotenoid content per unit volume⁶ in the tetraploid was 40 percent greater than in the diploid, but in terms of gene number per cell there was more than a twofold increase, as will be seen from the section on Cell-Volume Relations.

The tetraploid white-endosperm strain and the diploid from which it was derived contained appreciable amounts of carotenoids including beta-carotene and cryptoxanthin, which are precursors of vitamin A (table 1). In these strains, doubling the number of chromosomes and genes caused a decrease of 19 percent in carotenoid content. These white strains, which are designated Tetraploid White and Diploid White, originated from a cross between an inbred line of an early flint corn commonly known as Spanish Flint and an inbred line of white Argentine Flint corn. Other white-endosperm types of diploid corn, including White Rice popcorn, Yankee Cheat flour corn, and Onondaga White Dent corn, had a lower carotenoid content than the Diploid White corn (table 1). Alvord White Cap Dent, a yellow dent corn with a white crown, was also very low in total carotenoids.

In all of the tetraploid strains investigated, the weight of the individual kernels was approximately 50 percent greater than that of the

⁶ Since approximate measurements showed no significant difference in the density of the diploid and tetraploid kernels, the carotenoid content for unit weight, i. e., per gram of dry meal, is also the carotenoid content per unit volume.

related diploid. This percentage increase in kernel size is characteristic of most strains of tetraploid corn (11).

The commercial varieties and inbred lines of diploid yellow corn that were analyzed exhibited a wide range of values for total carotenoid content, as indicated in table 1. The highest value (0.0639 mg. per gram) was obtained from an inbred strain of Cornell 11, and the lowest values were from Dutton Flint (0.0144 mg.) and Golden Bantam sweet corn (0.0071 mg.). The inbred line of Cornell 11 had more than four times as much carotenoid as the Dutton Flint.

It was noted that the yellow appearance of the grain was not a reliable criterion of carotenoid content. For most of the commercial varieties there was a positive correlation between intensity of endosperm color and the amount of carotenoids present in the meal. Golden Bantam sweet corn was a conspicuous exception. This is a rich yellow sort, but it has the lowest carotenoid content of any of the yellow kinds that were compared. However, Argentine Yellow Flint, a very deep orange-yellow type, had a higher carotenoid value than the deep-yellow dent varieties Cornell 11 and Leaming. Among the inbred lines there was no consistent relation between endosperm color and total carotenoids. A deep-yellow Dutton Flint inbred had only about half as much total carotenoid as a medium-yellow inbred line of Luces Favorite, the values for the two lines being 0.0144 mg. and 0.0270 mg., respectively. One inbred line of Bloody Butcher had a rather low carotenoid content, while a second one had more than twice as much. The highest carotenoid value that was obtained for any inbred line or commercial variety was that of a Cornell 11 inbred, which had 0.0639 mg. of carotenoid per gram of dry meal, as compared with 0.0282 mg. for the commercial Cornell 11 variety from which it originated. However, the color of the endosperm was essentially the same in the inbred and in the parent variety. These results, demonstrating a lack of correspondence between carotenoid content and intensity of endosperm color, are in agreement with the results recently reported by Johnson and Miller (3, 4).

The commercial double-cross hybrid W29-3 and its parent lines were analyzed to determine the influence of hybridization on carotenoid content. In the production of this hybrid,⁷ the parent inbred lines were combined as follows: (W36-3 \times W36-4) \times (W36-2 \times W36-1), the first-named line being the seed parent of each hybrid combination. Since the endosperm tissue in which the carotenoids of the kernel are localized is triploid and originates from the combination of two sets of chromosomes and genes from the seed parent with one set from the pollen parent, the influence of the seed parent should be twice as great as that of the pollen parent in determining the carotenoid content of the hybrid, provided the genes affecting carotenoid content act in the cumulative manner described by Mangelsdorf and Fraps (8) for the yellow endosperm genes. The value for the single cross (W36-2 \times W36-1), involving a white-endosperm type, Onondaga White Dent, and a medium-yellow type, Luces Favorite, is in close agreement with the expected value, being nearer that of the seed parent than that of the pollen parent. The other single cross (W36-3 \times W36-4), which involved two deep-yellow endosperm types,

⁷ WIGGANS, R. G. Unpublished data.

Cornell 11 and Bloody Butcher (the yellow endosperm of this variety is obscured by the presence of red pericarp color), had more total carotenoids than the seed parent. This is of special interest since the seed parent, Cornell 11, had the highest value of any of the diploid types that were analyzed. The increase in the hybrid may have been due to the complementary action of additional genes for yellow contributed by the two parents. The plausibility of this explanation is strengthened by the recent discovery that yellow endosperm color in maize is dependent on the interaction of the dominant allelomorphs of two or more genes (9). The double-cross hybrid W29 3 had a somewhat lower carotenoid content than the expected value.

The extreme variation in the carotenoid content of inbred lines of yellow corn, including both the active provitamin A fraction and the inactive fraction, emphasizes the importance of carotenoid determination to evaluate the feeding quality of these strains and their hybrids. The carotenoid content of the Cornell 11 inbred line, which had the highest carotenoid value of the five yellow inbred lines that were analyzed, was more than four times as great as that of the Dutton Flint inbred, which had the lowest value of these five lines. Since endosperm color is not a reliable criterion of carotenoid content, except within very broad limits, chemical analyses or biological tests with animals are necessary to determine at all accurately the carotenoid value of a given sample of yellow corn. Furthermore, the results obtained from the analyses of strains of white corn indicate that some of them, as for example, Diploid White, possess sufficient provitamin A carotenoids to be detected by animal-assay experiments. However, the animal experiments that have been performed (2, 8, 14) have failed to demonstrate any significant vitamin A potency of white corn.

CELL-VOLUME RELATIONS

Chromosome doubling ordinarily results in an increase in cell size commensurate with the increase in nuclear volume caused by the presence in the nucleus of the double number of chromosomes. Autopolyploids of recent origin invariably have larger pollen grains and larger stomata than the forms with lower chromosome number from which they originated, but not so much is known about cell-volume relations in many other parts of the plant, as, for example, in the endosperm. Since the endosperm of the corn kernel is a simple tissue made up of relatively homogeneous cell components, it is favorable material for a study of the influence of chromosome doubling on cell volume.

At the present time very little is known about the influence of the changed conditions of cell volume and gene number per cell upon the action of specific genes following chromosome doubling. In diploid organisms certain genes exhibit differential quantitative action, while others do not, under conditions in which cell-volume relations remain relatively constant. In autotetraploids both cell size and gene number per cell are greater than in the parental diploid, thus creating changed conditions under which the action of the genes may differ from their known action in the diploid organisms. An investigation of cell-volume relations in diploid and tetraploid corn was undertaken to determine the extent to which the concentration of the genes for yellow

endosperm influenced the development of the character in question, i. e., the carotenoid content of the corn meal. This could be accomplished, since it was readily possible to determine cell volumes, and the degree of development of the character could be accurately determined by quantitative chemical and photometric analyses.

Since there is a twofold increase in the number of genes per cell in the tetraploid as compared with the diploid, the same concentration of the genes per unit volume will be maintained in the tetraploid if the volume of the cells is also doubled. If cell volume is not doubled, the concentration of the genes per unit volume will be increased in the tetraploid; if cell volume is more than doubled, there will be a corresponding decrease in gene concentration.

Computations of endosperm cell volume were made from the tissue in the central region of the endosperm and from the peripheral (aleurone) layer of cells in the midabgerminal region of mature kernels that in size and shape were representative of the diploid and tetraploid strains. In the middle region of the endosperm the cells were irregular in outline but isodiametric in longitudinal and cross sections. The relative volume of the cells in this region was computed by treating the cells as spheres whose areas were the areas of the cells in section view, areas being procured from planimeter measurements. The shape of the peripheral cells was essentially rectangular in both longitudinal and cross sections, their width when viewed in cross section of the kernel being somewhat less than their depth when viewed in longitudinal sections of the kernel. The volume of the aleurone cells was computed by multiplying together their three dimensions procured from the cross and longitudinal sections. Photomicrographs of representative regions of the endosperm from which cell measurements were taken are reproduced in plates 1 and 2.

For determining the mean cell volume in the two regions of the endosperm, five groups of six to eight contiguous cells were measured in each of four diploid and four tetraploid kernels selected as representative of the two strains. The values for cell volume obtained by this method of measuring all of the members of a given group of cells were somewhat lower than the true values, since the plane of section ordinarily was not median for all of the cells measured. Thus the maximum dimensions of all of the cells were not procured. The values are relative, not absolute, and provide an adequate basis only for a comparison of volume relations in the two kinds of corn. The computations of cell volume are given in table 2, including the means for each kernel and the means for all of the measurements from the two regions of the diploid and tetraploid kernels, together with their standard errors.

From the data presented in table 2 it is apparent that the endosperm cells in the tetraploid were very much larger than those of the diploid, the ratio of their volumes being essentially 3.6:1 in both the peripheral aleurone layer and in the central region. This is a much greater increase than has been reported in other studies of cell-volume relations following chromosome doubling. Nuclear volume in the same cells, as estimated from a limited number of measurements, was in the ratio of approximately 2.5:1, indicating that the ratio of chromosome numbers was 2:1 as expected in the endosperm of the diploid and tetraploid kernels that were examined.



Photomicrographs of endosperm tissue from the central region of the kernel in (A) diploid and (B) tetraploid corn taken at the same magnification. $\times 260$.



Photomicrographs of the aleurone layer and adjacent tissues from kernels of (A) diploid and (B) tetraploid corn taken at the same magnification. Longitudinal sections from the midabgerminal region of the kernel: *al*, Aleurone; *en*, endosperm; *p*, pericarp. $\times 260$.

TABLE 2.—Comparisons of cell volume¹ in the endosperm of diploid and tetraploid corn

Kernel No.	Central region		Aleurone layer	
	2n	4n	2n	4n
	<i>Cubic milli- meters</i>	<i>Cubic milli- meters</i>	<i>Cubic milli- meters</i>	<i>Cubic milli- meters</i>
1	175, 478 163, 051 153, 710 170, 673 163, 051	645, 068 708, 896 549, 181 418, 469 755, 536	5, 155 6, 380 6, 730 5, 085 4, 655	18, 380 20, 950 15, 700 19, 660 17, 420
Mean	165, 193	615, 430	5, 601	18, 422
2	157, 960 148, 036 145, 132 138, 685 180, 374	448, 943 410, 405 349, 585 413, 081 571, 698	4, 245 6, 115 4, 490 5, 360 5, 730	21, 160 20, 670 22, 600 23, 740 31, 280
Mean	154, 037	438, 742	5, 188	23, 890
3	119, 560 118, 036 141, 118 126, 108 188, 455	469, 084 397, 197 386, 833 693, 150 575, 773	6, 360 5, 970 7, 120 6, 400 6, 365	18, 080 19, 290 20, 820 21, 310 23, 800
Mean	138, 655	504, 407	6, 443	20, 660
4	143, 285 127, 342 152, 216 137, 010 128, 782	610, 112 560, 238 490, 720 701, 160 530, 272	4, 870 4, 465 6, 020 5, 250 5, 830	21, 240 18, 110 17, 260 19, 060 20, 750
Mean	137, 907	578, 500	5, 287	19, 284
Mean of all measurements	148, 948 ±4, 527	534, 269 ±26, 850	5, 630 ±179	20, 563 ±739
Ratio	1 : 3.58		1 : 3.65	

Volumes were computed in cubic millimeters from measurements procured at a magnification of 530 meters.

Although the individual cells of the tetraploid contained twice as many genes as did the cells of the diploid, the number of genes per unit volume was actually less in the endosperm of the tetraploid than in the diploid, owing to the pronounced increase in the volume of the cells of the tetraploid. Conversely, since the reduction in gene number per unit volume in the tetraploid was associated with a marked increase in carotenoid content per unit volume there was a very significant increase in the amount of carotenoid per gene elaborated by the tetraploid, and a still greater increase in the amount of carotenoid per cell. These proportional differences between the endosperm of the diploid and tetraploid strains are shown in table 3.

TABLE 3.—Proportional differences between the endosperm of diploid and tetraploid strains of yellow corn

Item	Diploid	Tetraploid	Item	Diploid	Tetraploid
unit volume	1	3.6	Genes per unit volume	1	0.55
	1	1.4	Carotenoid per cell	1	5
	1	2	Carotenoid per gene	1	2.5

The disproportionate increase in the size of the endosperm cells of the tetraploid, which were 3.7 times as large as those of the diploid, may have been due to their hexaploid constitution with which was associated a retarded nuclear and cell-division activity and a compensating increase in cell enlargement. Evidence in support of this view is furnished by octaploid corn plants, which invariably are much reduced in stature and have cells that are relatively very large and few in number. However, the kernels of the tetraploid were 50 percent larger than those of the diploid, and this increase was roughly proportional for the constituent parts of the kernel, including the endosperm, embryo, and pericarp. It is not known to what extent the increased size of the hexaploid endosperm was conditioned by the influence of other parts of the kernel that were tetraploid in chromosomal constitution.

The fact that in the tetraploid there was more than a twofold increase in the amount of carotenoid per gene could be interpreted to mean that a doubling of the number of genes per cell more than doubles the efficiency of each gene. But it might be argued that the endosperm is primarily a storage tissue and that the amount of carotenoid which it contains is conditioned by relationships existing in other tissues of the plant where carotenoids are being synthesized. For example, the leaf tissue contains appreciable amounts of the same carotenoids that are present in the endosperm and it is conceivable that the endosperm serves merely as a storage organ for a portion of the carotenoids synthesized by the leaves. However, it was shown by Johnson and Miller (3) that the amount of carotenoid in the leaf tissue of white- and yellow-endosperm sister lines was essentially the same, but the endosperm of the white lines contained very small amounts of carotenoid. Their work also substantiated the earlier results of Mangelsdorf and Fraps (8) to the effect that there is a positive correlation between the number of dominant genes for yellow and the amount of carotenoids in the endosperm of ordinary diploid corn. Thus it appears that the carotenoid content of the endosperm tissue is determined by the number of dominant genes for yellow endosperm present in the tissue rather than by conditions existing elsewhere in the plant; furthermore, from the results of the present investigation it may be concluded that the number of genes per cell unit is of more importance than the number of genes per unit volume in determining the amount of carotenoids present in the endosperm.

DISCUSSION

The results reported here have demonstrated a percentage increase following chromosome doubling in the carotenoids of yellow corn, and a percentage decrease in the carotenoids of white corn. Obviously, when there is a percentage increase in some of the substances in the corn kernel there must be a corresponding percentage decrease in other components, and vice versa. The effect of chromosome doubling on the relative amounts of other important constituents of the corn kernel, such as carbohydrates, proteins, and fat, was not determined in this investigation.

The percentage increase in the carotenoid content of the tetraploid yellow corn is interpreted as being due to a cumulative action of the dominant genes for yellow endosperm color. Since these dominant genes were not present in the white corn in an effective combination to

produce well-developed endosperm color, cumulative action was lacking in the tetraploid white corn and there was a resultant percentage decrease in carotenoids.

These results suggest that there are two categories of gene action in autotetraploids: (1) Cumulative gene action, which yields percentage increases and accounts for the distinctive traits of autotetraploids other than those that may be attributed directly to the presence of an increased number of chromosome sets, and (2) noncumulative gene action, which yields percentage decreases when the percentages of other constituents are increased. The significance of this classification can be understood better in the case of corn carotenoids if the quantity of pigment is expressed in amount per cell rather than in percentage. In the tetraploid yellow corn, owing to cumulative gene action, there was a fivefold increase in the amount of carotenoids per cell. In the tetraploid white corn, if it is assumed that the cell-volume relations in the diploid and tetraploid were the same as in the yellow corn, there was an increase in the amount of carotenoid per cell as a result of chromosome doubling; but this increase was very much less than in the yellow corn, owing to the absence of cumulative gene action. Further study of the differences that distinguish chromosome-doubled strains from their parent strains is needed, based on analyses of individual traits which may or may not be conditioned by cumulative gene action.

The relative importance of autotetraploids as horticultural and crop plants will be determined by the extent to which desirable traits are accentuated or produced by chromosome doubling without accentuating or producing undesirable traits. In terms of gene action, this means that chromosome-doubled strains of cultivated plants may have increased value if their desirable traits are controlled by genes that function in a cumulative manner to yield significant percentage increases like those reported here for the carotenoids of yellow corn. The fact that most of the more important crop plants are polyploids suggests that cumulative gene action has been an important determining factor in the evolution of cultivated plants.

SUMMARY AND CONCLUSIONS

Doubling the number of chromosomes in pure yellow corn caused a 40-percent increase in the content of carotenoid pigment.

The active provitamin A fraction of the carotenoids, including beta-carotene and cryptoxanthin, was increased in the tetraploid yellow corn approximately in proportion to the increase in total carotenoid pigment.

The volume of the endosperm cells of the tetraploid was approximately 3.6 times the volume of the endosperm cells of the diploid.

The increase in the cell volume and the carotenoid content of the endosperm in the tetraploid yellow corn resulted in a fivefold increase in the amount of carotenoid per cell.

The genes for yellow endosperm exerted a cumulative action following chromosome doubling. In the individual endosperm cells of the tetraploid the amount of carotenoid elaborated per gene was 2.5 times as great as in the individual cells of the diploid, even though there was a greater concentration of genes per unit volume in the diploid than in the tetraploid.

Doubling the number of chromosomes in white corn decreased the carotenoid content 19 percent. With respect to carotenoid content there was no cumulative gene action in the white corn.

The carotenoid content varied widely among different commercial varieties, inbred strains, and hybrids of ordinary diploid yellow corn. The inbred line with the highest carotenoid content had more than four times as much carotenoid as the line with the lowest carotenoid content.

The yellow appearance of the kernel was not a reliable criterion of carotenoid content.

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THE EFFECT OF ADDING BLACKSTRAP MOLASSES TO A LAMB-FATTENING RATION¹

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INTRODUCTION

Considerable interest was manifested in the substitution of blackstrap molasses for a part, or all, of the grain in livestock rations during the drought years of 1934 and 1936. Ferrin² used molasses with corn and oats in fattening hogs and found that oats and molasses had a comparatively high value as compared with corn and molasses. Thompson and Hillier³ experienced similar results in feeding swine on rations containing ample protein. Blizzard and Taylor⁴ likewise found that oats and molasses made a more valuable ration for fattening steer calves than did corn and molasses when both rations were properly supplemented with cottonseed meal.

These results suggest that possibly oats and molasses supplement each other in the ration to a better advantage than corn and molasses. Forbes, Braman, et al. (5) have pointed out that the feeding value of a feed may vary widely depending upon the combination in which it is fed.

Patterson and Outwater (12) studied the influence of blackstrap molasses in a steer ration and reported that the molasses increased not only the palatability but also the digestibility of the ration. Lindsey and Smith (9) could not confirm these conclusions in a digestion trial with mature sheep and concluded that the digestion of all nutrients except fat was hindered when appreciable amounts of blackstrap molasses were included in the ration.

Snell (16) investigated the effect of molasses on the digestibility of steer rations. He used three yearling steers in each of two trials and found that various levels of molasses did not consistently alter the digestion of the nutrients in an ordinary steer ration consisting of corn, cottonseed meal, and grass hay.

A series of digestion trials was designed with lambs to find whether the addition of large amounts of molasses to an ordinary lamb-fattening ration would decrease the digestibility of the nutrients in the ration. The results of these trials are reported in this paper.

PROCEDURE

Four wether lambs were used in each of 3 separate trials, a total of 12 lambs being used in the study. All of the lambs were crossbred. One parent of each was a purebred Rambouillet and the other a purebred of either the Hampshire, Shropshire, or Southdown breeds. The lambs were all selected from the college flock and were thrifty, and hearty eaters. Each lamb of the 3 groups weighed approximately

¹ Received for publication July 26, 1939.

² FERRIN, F. E. CANE MOLASSES IN HOG RATIONS. Minn. Agr. Expt. Sta. Mimeographed Rpt. H-70. 1937.

³ THOMPSON, C. P., and HILLIER, J. C. BLACKSTRAP MOLASSES AS A SUBSTITUTE FOR CORN AND OATS IN FATTENING RATIONS FOR HOGS. Okla. Agr. Expt. Sta. Mimeographed Cir. 8. 1938.

⁴ Italic numbers in parentheses refer to Literature Cited, p. 71.

80 pounds at the start of the 3 trials, and all lambs gained during the experimental period.

The lambs were each fed test rations for a 10-day preliminary period and then placed in metabolism cages for a 10-day collection period. The cages were similar to those described by Forbes (4). The lambs ate readily after they became accustomed to the cages. The lambs usually gained slightly while in the cages on collection, but did not gain as much as they did in the pens during the preliminary periods.

The procedure used was the same as that previously reported by Briggs (3). Each lamb was fed the daily rations given in table 1. The feeds were carefully weighed on a scale sensitive to 0.1 gm. Each lamb was fed in an open bucket while in the preliminary pen, but in a specially constructed feeder while in the cage. The lambs were fed at approximately the same time each morning and evening and received one-half the daily ration at each feeding.

The corn and oats were fed whole while the alfalfa hay was ground in a hammer-mill and forced through a $\frac{1}{4}$ -inch-mesh screen. The blackstrap molasses was weighed, diluted with water, and used to moisten the ration. The entire ration was thoroughly mixed before feeding.

Feces collections were made each morning at the same hour. Each day's collection was dried separately for 24 hours over an electric heater. The collection was then weighed and sealed. At the close of the 10-day collection period the dry feces were placed in a large container, thoroughly mixed, and representative samples were taken for chemical analysis.

TABLE 1.—Quantitative composition (grams) of the daily rations used in digestion trials with lambs

Component	Ration—			
	A	B	C	D
Corn (yellow).....	460	230		
Oats.....			460	230
Alfalfa hay.....	454	454	454	454
Blackstrap molasses.....		230		230

The feeds given to lambs 1a, 2a, 3a, and 4a during the first digestion trial are shown in table 2. The trial was conducted during the interval from May 10 to August 26, 1937. The feeds used were representative of those produced during the dry growing season of 1936. Perhaps the extreme drought during the growing season caused the corn to be below normal in fat content. This analysis was checked and rechecked. In all other respects the feeds seemed to be normal in chemical composition. The market grades of the feeds used are also shown in table 2. The feeds used in the second trial were grown during the more normal reason of 1937. The composition and grade of these feeds are given in table 2 and they were consumed by lambs 1b, 2b, 3b, and 4b. Lambs 1c, 2c, 3c, and 4c were used in the third trial and were fed the feeds listed, with their chemical composition and grade, in table 2. These feeds were grown during the season of 1938. The market grades of the feeds used in these studies were secured through the courtesy of the Bureau of Agricultural Economics, United States Department of Agriculture.

RESULTS

Table 3 presents the apparent digestion coefficients secured in each trial. Each of the 12 lambs was fed consecutively on each of the 4 rations studied.

TABLE 2.—Percentage composition and commercial grade of feeds used in three digestion trials with lambs

TRIAL 1, MAY 10 TO AUGUST 26, 1937

Feed	Water	Protein	Fat	Crude fiber	Ash	Nitrogen-free extract	Grade
Corn.....	14.01	9.90	1.91	2.21	1.55	70.42	No. 5
Oats.....	9.66	11.71	3.74	10.24	3.92	60.73	No. 2
Alfalfa hay.....	10.31	15.14	.89	26.64	7.87	39.15	No. 2
Molasses.....	29.10	2.40			8.35	60.15	

TRIAL 2, FEBRUARY 25 TO JUNE 14, 1938

Corn.....	13.89	9.89	3.19	2.14	1.29	69.60	No. 2
Oats.....	10.75	13.88	3.30	10.24	3.36	58.29	No. 2
Alfalfa hay.....	11.25	13.98	.89	27.57	7.73	38.58	No. 2
Molasses.....	31.72	2.00			9.40	56.88	

TRIAL 3, JANUARY 10 TO APRIL 14, 1939

Corn.....	12.29	10.17	3.61	1.94	1.06	70.93	No. 2
Oats.....	9.49	12.38	4.46	14.38	3.62	55.67	No. 2
Alfalfa hay.....	5.99	14.75	2.95	25.50	6.93	43.88	No. 2
Molasses.....	35.40	1.62			9.22	53.76	

¹ Excess of shrunken kernels.

TABLE 3.—Effect of blackstrap molasses on the apparent digestibility (percent) of lamb rations

Lamb No.	Protein digestion coefficient for ration—				Fat digestion coefficient for ration—				Crude fiber digestion coefficient for ration—				Nitrogen-free extract digestion coefficient for ration—			
	A, corn, alfalfa	B, corn, alfalfa, molasses	C, oats, alfalfa	D, oats, alfalfa, molasses	A, corn, alfalfa	B, corn, alfalfa, molasses	C, oats, alfalfa	D, oats, alfalfa, molasses	A, corn, alfalfa	B, corn, alfalfa, molasses	C, oats, alfalfa	D, oats, alfalfa, molasses	A, corn, alfalfa	B, corn, alfalfa, molasses	C, oats, alfalfa	D, oats, alfalfa, molasses
1a.....	77.2	65.8	73.7	69.1	34.6	18.5	62.6	34.9	42.6	41.1	50.5	39.0	87.1	84.7	78.1	80.8
2a.....	73.0	73.4	74.7	63.5	35.4	21.9	57.0	47.5	52.5	29.6	50.4	39.1	85.1	88.3	80.2	79.9
3a.....	77.0	70.7	74.5	71.2	53.6	16.6	64.4	24.5	38.4	37.2	54.1	53.2	86.6	85.6	79.7	78.4
4a.....	78.9	72.0	77.8	67.7	55.8	20.0	76.0	37.9	51.1	41.9	46.1	40.0	86.2	86.0	81.8	81.1
Average.....	76.5	70.5	75.2	67.9	44.8	19.2	65.0	36.2	46.1	37.4	50.3	43.0	86.2	86.1	79.9	80.0
1b.....	66.0	63.0	73.1	67.1	49.0	27.0	64.4	45.0	46.0	45.9	52.1	45.7	83.6	82.2	80.0	77.0
2b.....	65.8	67.9	75.4	72.2	58.7	28.5	63.8	53.3	51.0	51.3	56.4	48.2	83.2	70.6	76.0	76.2
3b.....	67.5	60.4	75.4	68.7	48.7	45.6	62.9	37.1	46.4	43.6	54.3	48.7	84.3	81.9	78.3	74.5
4b.....	66.1	63.0	76.3	71.3	46.2	24.7	68.9	54.7	40.5	52.9	57.8	51.3	82.9	81.3	79.0	78.0
Average.....	66.3	63.8	75.0	69.8	50.6	30.2	65.0	47.5	46.0	48.4	55.1	48.5	83.5	79.0	78.3	76.4
1c.....	63.3	55.8	65.8	69.2	71.2	60.0	66.0	74.5	33.2	36.4	47.5	52.9	84.6	84.9	79.2	83.7
2c.....	64.2	61.4	69.3	63.1	68.3	60.7	80.5	65.7	30.5	25.2	45.1	43.7	85.5	83.8	76.4	83.6
3c.....	62.5	54.3	63.4	64.9	76.6	65.8	71.5	72.5	33.9	45.6	43.9	39.6	83.5	86.0	79.8	82.2
4c.....	62.8	68.0	66.2	62.1	66.3	61.5	81.2	68.1	38.6	37.3	45.5	42.5	86.5	85.9	76.2	80.7
Average.....	63.2	59.9	66.2	64.8	70.6	62.0	74.8	70.2	34.0	36.1	45.5	44.7	85.0	85.3	77.9	82.5
Average coefficient (3 trials).....	68.7	64.7	72.1	67.5	55.4	37.1	68.3	51.3	42.1	40.7	50.3	45.4	84.9	83.5	78.7	79.7

DISCUSSION

The data presented in table 3 were analyzed by the method presented by Snedecor (15). The digestion coefficients of each nutrient were studied separately. Since the 12 lambs had been studied in 3 different periods, with different feeds, considerable difference existed among the coefficients of the separate periods. The difference between periods was highly significant in the case of protein, fat, and fiber coefficients, and it was therefore necessary to remove the variation between periods in the analysis of these data.

THE EFFECT OF BLACKSTRAP MOLASSES ON PROTEIN DIGESTION

The apparent digestion coefficients of the protein in the rations are shown in table 3. During the three trials, the replacement of one-half the corn ration for an equal weight of molasses lowered the coefficient of digestibility of protein in a corn-alfalfa hay ration 4 percent.

This difference did not prove to be significant when the effect of the periods was removed. In each separate trial, the digestibility of the ration had been lowered by molasses, but considerable difference existed in the digestion efficiency among the periods. This discrepancy was highly significant.

The digestibility of the protein in the oats-alfalfa ration did not vary much between periods. In two of the three trials, the average coefficient was considerably lower when blackstrap molasses was included in the ration while there was less decrease in the third trial. Lambs 1c and 3c gave slightly higher coefficients when the concentrates consisted of one-half blackstrap molasses. The average apparent digestion coefficient of protein was lowered 4.6 percent, and the difference was highly significant.

In a digestion study with dairy cows Williams (17) found that the addition of blackstrap molasses to the ration had a variable effect on digestion coefficients, but that in general the addition of the molasses lowered the digestion coefficient of protein. An analysis of the digestibility of protein of the basal rations showed no significant difference. However, most of the lambs digested a slightly higher percentage of the protein from the oats-alfalfa ration than they did from the corn-alfalfa ration. This finding is in agreement with that of Morrison (10) who gives oats as having a slightly higher digestion coefficient for protein than corn.

THE EFFECT OF BLACKSTRAP MOLASSES ON FAT DIGESTION

A substitution of blackstrap molasses for one-half of the grain in a lamb ration decidedly lowered the digestibility of the fat in the ration. The coefficient of apparent digestibility of fat of a corn-alfalfa hay ration averaged 55.4 percent for the 3 trials. When one-half of the corn was replaced by an equal weight of molasses the coefficient dropped to 37.1 percent or a difference of 18.3 percent. This decrease was highly significant. In each of the 12 lambs studied the addition of molasses decreased the digestibility of the fat in the ration. The fat in the rations proved to be more digestible in each succeeding trial. This may have been due to the efficiency of the lambs, but probably was the result of the varying character of the feeds.

In a similar manner the replacement of one-half the oats in an oats-alfalfa hay ration with an equal weight of molasses decreased the digestibility of the fat in the ration of 10 of the 12 lambs. Lambs 1c and 3c in the third trial digested the fat in the ration more efficiently when molasses was present. Notwithstanding, the average of the 12 lambs gave the oats-alfalfa ration a fat digestion coefficient of 68.3 percent as contrasted with 51.3 percent when molasses was added to the ration. This difference was highly significant.

Perkins and Monroe (13) noted a depression in the digestion coefficients of the nutrients in a dairy-cow ration when blackstrap molasses was included. The digestion coefficient of fat was lowered more than that of the other nutrients in the low protein rations which they used.

Morrison (10) gives the fat digestion coefficient of corn as slightly higher than that of oats. The corn used in this study had an average fat content of 2.90 percent, and the oats an average fat content of 3.83 percent. The alfalfa contained an average fat content of 1.58 percent. The lambs gave 12.9 percent higher fat digestion coefficients for the oats-alfalfa hay ration than for the corn-alfalfa hay ration. This difference proved to be highly significant. A difference in digestion coefficients of only 1.4 percent would be anticipated in favor of the oats-alfalfa hay ration from using the digestion coefficients of Morrison in calculating the expected efficiency with which lambs would handle each ration. Oats are higher in fat than corn; and if the fat is also more completely digested in certain lamb-ration combinations, it raises the total digestible nutrients or energy value of a ration above that anticipated. This may in part explain the higher regard that practical shepherds have for the fattening value of oats as compared with the total digestible nutrient value commonly assigned them.

THE EFFECT OF BLACKSTRAP MOLASSES ON CRUDE FIBER DIGESTION

Sheep and other herbivorae consume large amounts of crude fiber in their rations. It is therefore important to know what effect ration variations may have on the digestion of this nutrient. The addition of molasses slightly lowered the digestibility of the fiber in both the corn-alfalfa hay ration and in the oats-alfalfa hay ration. However, neither of these differences was significant.

The lambs digested the fiber in the oats-alfalfa hay ration to a greater extent than that in the corn-alfalfa hay ration. This difference proved to be highly significant. This is perhaps another factor in favor of oats as compared with corn as a feed for sheep. The effect of a feed upon the digestibility of a ration is no doubt more important than the way the single feed is digested when fed alone or in combination with certain other feeds.

THE EFFECT OF BLACKSTRAP MOLASSES ON NITROGEN-FREE-EXTRACT DIGESTION

The nature of the computation of the nitrogen-free-extract content of a feed or feces makes it rather an unsatisfactory value to study in digestion trials. It would seem logical that an increase in the sugar content of a ration would raise the nitrogen-free-extract digestion coefficients. A substitution of blackstrap molasses for one-half the grain in the rations of lambs had no material effect on the nitrogen-free-extract digestion of the ration.

In the third trial all four lambs showed a highly significant increase in the digestion of nitrogen-free extract when molasses was added to

an oats-alfalfa hay ration. This same addition caused no increase in the same ration in the other two trials, but showed a slight decrease in the second trial. In no case did the addition of molasses make a significant alteration of the nitrogen-free-extract digestion of a corn-alfalfa hay ration.

The lambs ate all four of the rations with relish and changed readily from the nonmolasses to the molasses rations and vice versa. The lambs were fed less than a full feed so that their appetites were always keen, and the feed was cleaned up regularly. During each experimental period the lambs gained steadily in weight and seemed to store some fat on their bodies.

Armsby (1) referred to a condition of "depression of digestibility" in rations that contain an excess of carbohydrates as compared with protein in a ration. Schneider and Ellenberger (14) encountered a depression of 4.0 ± 0.9 percent in the digestibility of fat in a dairy-cow ration low in protein as compared with one high in protein. The energy levels were approximately the same in both rations. Hold-away and coworkers (6, 7) observed that unless adequate protein was present in a ration additions of apple pomace lowered the digestibility of the nutrients. Adding corn to a mixed-hay ration lowered the digestion coefficients of the protein, if a protein supplement was not used. The digestibility of the other nutrients was not materially changed by the addition of corn to the hay ration.

The nutritive ratios of the different rations used in this study varied, but all were as narrow as those suggested by Morrison (10) for the weight of lambs used. The computed nutritive ratio of the widest ration used was 1:7.24; Morrison's digestion coefficients were used in making these calculations. The lambs weighed an average of about 80 pounds each at the start of the experiments, and the average weight for the trial was approximately 90 pounds. It would not appear that the lambs used in this study were lacking in protein in view of modern feeding standards.

Nakamura (11) has shown that the administration of calcium lactate resulted in an increased excretion of fat and fatty acids in the feces of dogs. Likewise, Irwin, Weber, and Steenbock (8) have shown that large amounts of calcium and potassium chlorides will decrease the digestion of fat in the ration of the rat. Molasses is high in both calcium and potassium salts. It is therefore probable that the laxative effect of molasses experienced by the lambs while on the ration to which molasses was added lowered the digestion of fat and other nutrients.

It is possible that concentrated amounts of sugar in the rumen might interfere with normal functions in that organ. This consideration is probably not important as fiber digestion was not materially influenced, but the digestion of protein and fat nutrients, usually credited to the lower parts of the alimentary canal, was noticeably affected.

The large amounts of molasses fed in this trial are in excess of those that would likely be fed in common practice except in cases where molasses might be considerably cheaper than other concentrates.

SUMMARY

In a series of digestion trials the inclusion of large amounts of blackstrap molasses lowered the digestibility of certain nutrients in a lamb

ration. Four lambs were used in each of three separate digestion trials. The lambs were placed on each ration for a preliminary period of 10 days. They were then placed in a metabolism cage and the feces recovered for a like period.

The substitution of 230 gm. of molasses for the same amount of corn in a corn-alfalfa hay ration lowered the digestion coefficients of crude protein, but the decrease was not significant. The digestibility of fat was lowered 18.3 percent which was highly significant. The average crude-fiber and nitrogen-free-extract coefficients were lowered very slightly but not in significant amounts.

The replacement of 230 gm. of oats with molasses in an oats-alfalfa hay ration lowered the digestion of protein the highly significant amount of 4.6 percent. The digestibility of fat was decreased 17 percent, a highly significant amount. When the three trials are considered together, the changes in the digestibility of fiber and nitrogen-free-extract are not significant. In the third trial the addition of molasses to the oats-alfalfa hay ration did increase, to a highly significant extent, the digestibility of the nitrogen-free-extract of the ration.

The lambs used in the study digested the fat and fiber in an oats-alfalfa hay ration more completely than the smaller amounts of fat and fiber in a corn-alfalfa hay ration. The difference was highly significant.

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JOURNAL OF AGRICULTURAL RESEARCH

VOL. 60

WASHINGTON, D. C., JANUARY 15, 1940

No. 2

A ROT OF WINTER QUEEN WATERMELONS CAUSED BY PHYTOPHTHORA CAPSICI¹

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INTRODUCTION

Winter Queen watermelons,² known also as winter watermelons, are found on the New York market during September and October. In limited quantities they meet the demand for a late-season watermelon, while many of smaller size are pickled whole and eaten as a delicacy by certain groups.

The annual unloads of Winter Queen watermelons on the New York market, from 1928, the first year that separate records were kept, to 1938, inclusive, were as follows:³

Year:	Carloads
1928	13
1929	11
1930	28
1931	36
1932	29
1933	27
1934	14
1935	16
1936	7
1937	3
1938	4

It will be seen that unloads increased from 13 carloads in 1928 to a peak of 36 in 1931 but thereafter decreased markedly so that by 1937 only 3 carloads were handled, and in 1938, 4 carloads. Although few in number during 1937 and 1938, the melons arrived in good condition, were well received by the trade, and brought encouraging returns on the market. It appears reasonable to expect that annual unloads will increase substantially over the low point reached in 1937 and 1938.

In September 1935 a carload of Winter Queens arrived at New York City with approximately 80 percent of the melons affected with a phytophthora rot. Although a field and local market decay of Iowa Belle watermelons, caused by a species of *Phytophthora*, had been reported from Arizona (2),⁴ this was the first record of a phytophthora rot of Winter Queen melons. The seriousness of the decay when

¹ Received for publication January 25, 1939. Cooperative investigation by the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Missouri Agricultural Experiment Station.

² The Winter Queen is a variety of watermelon (*Citrullus vulgaris* Schrad.) that is thought to have been introduced into this country by immigrants from the Volga region of the Union of Soviet Socialist Republics. The fruits are small, nearly round, approximately 9 to 9½ inches in diameter, and weigh about 10 to 15 pounds. Although a few melons of large size (11 to 12 inches in diameter) are packed for the market, most are of the smaller sizes, many being about 6 inches in diameter. The rind is greenish to light cream in color with faint irregular stripes of light green. The pulp is bright red, stringless, of fine texture, firm, and sweet. The seeds are small and black. The melon is grown in the cantaloup- and Honey Dew melon-producing section of Colorado, packed in flat crates similar to those used for Honey Dew melons, and shipped to the market in refrigerator cars without ice, either under standard ventilation or with vents open, to destination.

³ Data taken from the annual reports of the unloads of fruits and vegetables at New York City, issued in mimeographed form by the Bureau of Agricultural Economics, U. S. Department of Agriculture.

⁴ Italic numbers in parentheses refer to Literature Cited, p. 88.

first observed on the market suggested the advisability of learning more about its occurrence and economic importance. Periodic examinations were therefore made of these melons on the unloading and sales pier at New York during the remainder of the 1935 season as well as during the following three seasons. A study was likewise made of the identity and pathogenicity of the species of *Phytophthora* involved, the manner of infection of the melons, the spread of the disease from melon to melon within the container, and the temperature relations of the pathogen and of the disease. The present paper summarizes the results of these studies, made over a period of four marketing seasons, and makes available such information as has been obtained regarding the control of the disease.

REVIEW OF LITERATURE

Sherbakoff (13) in 1917 produced a decay of watermelon fruits by artificial inoculation with *Phytophthora parasitica* Dastur.⁵

A decay of watermelon fruits caused by *Phytophthora citrophthora* (E. H. and R. E. Smith) Leon., was reported from California (4) in 1929. Squash and pumpkin fruits were likewise found infected.

Brown and Evans (2) in 1933 described a fruit rot of Iowa Belle watermelons that was responsible for the loss of 40 to 50 percent of the crop in a 10-acre field near Marana, Ariz. The pathogen, *Phytophthora cactorum* (Cohn and Leb.) Schroet., was found capable, through artificial inoculation, of causing a similar decay of cucumber fruits.

Ramsey, Wiant, and Link (10, p. 62), in 1938 made brief mention of the decay of Winter Queen watermelons described in the present paper.

Reports of phytophthora rots of other cucurbit fruits include the following:

Curzi (5) produced a decay of the fruits of squash and those of *Bryonia dioica* Jacq. by artificial inoculation with *Phytophthora capsici* Leon.⁶

Drechsler (6) isolated a species of *Phytophthora* with prominently papillate sporangia from a Honey Dew melon found on the Washington, D. C., market after shipment from either California or Colorado. He produced a similar decay by artificial inoculation with *P. cactorum*, *P. citrophthora*, and *P. parasitica*.

Tompkins et al. (14) successfully inoculated watermelon and citron fruits with *Phytophthora drechsleri* Tucker from sugar-beet roots. The fungus was found capable of infecting fruits of summer squash through the unbroken skin as well as through wounds.

Tompkins and Tucker (15) described a field decay of Honey Dew melons in California, caused by *Phytophthora capsici*. The disease was transmitted by artificial inoculation to fruits of cucumber, pumpkin, squash, Klondike watermelons, and a number of types of muskmelon including cantaloups and Casaba, Honey Ball, Honey Dew, and Persian melons. *P. drechsleri* was involved to a slight extent. These authors (16) also found that fruits of pumpkin, watermelon,

⁵ Described by Sherbakoff as a new species, under the name *P. terrestris*, (corrected to *terrestris*) but considered by Butler (3) and other students of the genus to be identical with *P. parasitica*.

⁶ Described by Curzi as a new species, *P. hydrophila*, but considered by Tucker (17) to be identical with *P. capsici*.

and cucumber could be successfully inoculated through the unbroken skin with *P. cryptogea* Pethybr. and Laff. from China-aster.

Wiant (18) reported studies made on the New York City market of a phytophthora rot of Honey Dew melons from California, Colorado, and Chile. A similar decay of cantaloups and Honey Ball melons was produced by artificial inoculation.

Kreutzer (7) described a field rot of cucumber fruits causing a nearly complete loss of the crop in an 8-acre field located near Rocky Ford, Colo. He stated that the pathogen was probably *Phytophthora capsici*. Fruits of the squash were successfully inoculated with the fungus.

THE DISEASE

OCCURRENCE AND IMPORTANCE

Phytophthora rot of Winter Queen watermelons was first observed in a carlot unloaded at New York in early September 1935. An average of approximately 80 percent of the melons in the car were partly decayed, and in many of the crates all were infected. The decayed spots were mostly over 3 inches in diameter, with from one to three lesions per melon. The entire carlot was rejected for sale and was disposed of as garbage.

This car was shipped from Rocky Ford, Colo., 5 days previous to the date of unloading at New York. On the same day two other cars were shipped from Manzanola, Colo. These were unloaded at New York 5 and 7 days, respectively, after the date of loading. In the first of these from 15 to 50 percent of the melons were affected with phytophthora rot, with an average of 25 percent for the carlot. Approximately 15 percent of the melons in the second of these two carlots likewise were found to be in early to advanced stages of decay with phytophthora rot, while in a group of 73 crates nearly 40 percent of the melons were so affected.

Although observations were made on the condition of Winter Queen watermelons at the unloading and sales pier from time to time during the remainder of the 1935 season, only in the three carlots noted above was phytophthora rot found present. In this connection it is of interest to note that certificates issued by the Bureau of Agricultural Economics, United States Department of Agriculture, on eight additional carlots and two mixed carlots containing some Winter Queens indicated that four carlots (including one mixed carlot) were free of decay, while in six carlots (including one mixed carlot) from 1 to 8 percent (averaging 5 percent) of the melons were decayed. The decay was reported as alternaria rot and as an early stage of rhizopus soft rot. No inspections were made of the remaining three or four carlot equivalents of that season.

The unloading dates of the seven cars arriving at New York during the season of 1936 were scattered through a 5-week period; consequently, observations were limited. In one car, however, phytophthora rot was found, but the percentage of melons so affected was not determined. An examination of inspection reports on seven carlots (including one mixed lot), made either by the Bureau of Agricultural Economics or by the Railroad Perishable Inspection Agency, indicated 15 percent of decay in one carlot and from 1 to 5 (averaging 3.5) percent of decay in the other six carlots (including

one mixed lot). As during the previous season, the decay was for the most part recorded as alternaria rot and rhizopus soft rot.

No phytophthora rot was found in the seven carlots unloaded during 1937 and 1938. In fact, practically no decay of any kind was observed.

The results of the observations made over the four-season period may be summarized by stating that, out of a total of 30 carlots, phytophthora rot was definitely known to have occurred in 4, where it was responsible for from 5 percent or less to nearly a total loss of the melons. It definitely was not present in the 7 carlots of 1937 and 1938. Of the remaining 19 carlots, inspections by others on 15 to 16 carlots (or carlot equivalents, because of the mixed loads) suggest that, if phytophthora rot occurred at all, it was not responsible for heavy losses. However, this does not preclude the possibility that the decay may have been present in small amounts in some of the carlots without being recognized. It would appear that under certain conditions phytophthora rot may constitute a problem of the greatest importance in carlots of Winter Queen watermelons shipped to eastern markets.

SYMPTOMS

The earliest symptom of phytophthora rot is the development of either a slight water soaking or a reddish-brown ("vinaceous-tawny")⁷ discoloration of the rind about the point of inoculation. When the water-soaking type of discoloration prevails, the lesion as it advances may become "grape green," "deep grape green," "deep olive-buff," "dark olive-buff," or "citrine drab" in color. Frequently there is little evidence of water soaking during the early stages of decay. Then the affected rind assumes colors of brown ("russet," "sayaal brown," "snuff brown," "Saccardo's umber," or "mars brown").

The lesions are at first irregular in pattern (fig. 1, *A*). By the time they have attained a diameter of approximately 1 inch they become nearly circular in shape (fig. 1, *B*), although the margin may still be irregular. There may be one or several (fig. 1, *C*) lesions on a melon. When more than one occurs, adjacent lesions may coalesce in part as they enlarge. At the same time, isolated lesions assume a more nearly circular shape and their margins become regular and distinct (fig. 2, *A*). Frequently the lesions have a mottled appearance (fig. 2, *A*), or alternating concentric bands of several colors may be present (fig. 1, *C*).

In advanced stages of decay the white mycelium of the causal fungus develops on the affected areas of the melon (figs. 2, *A* and *B*; 3, *A* and *B*). This is first evident at the center of the lesion (fig. 2, *A*) and finally appears over all of the surface except that of the advancing edge of the lesion (figs. 2, *B*; 3, *A*). Such melons, confined within a closed moist chamber, develop a luxuriant white cottony mold growth. Under the conditions usually prevailing on the market, however, the mold does not grow out much from the surface but tends rather to become matted down against the moist tissues of the lesion.

The affected tissues early become somewhat softer than normal but retain their form for some time. Eventually there is shriveling and wrinkling with formation of creases in the diseased rind (figs. 2, *A*;

⁷ All color designations in quotation marks in this paper are according to Ridgway (11).

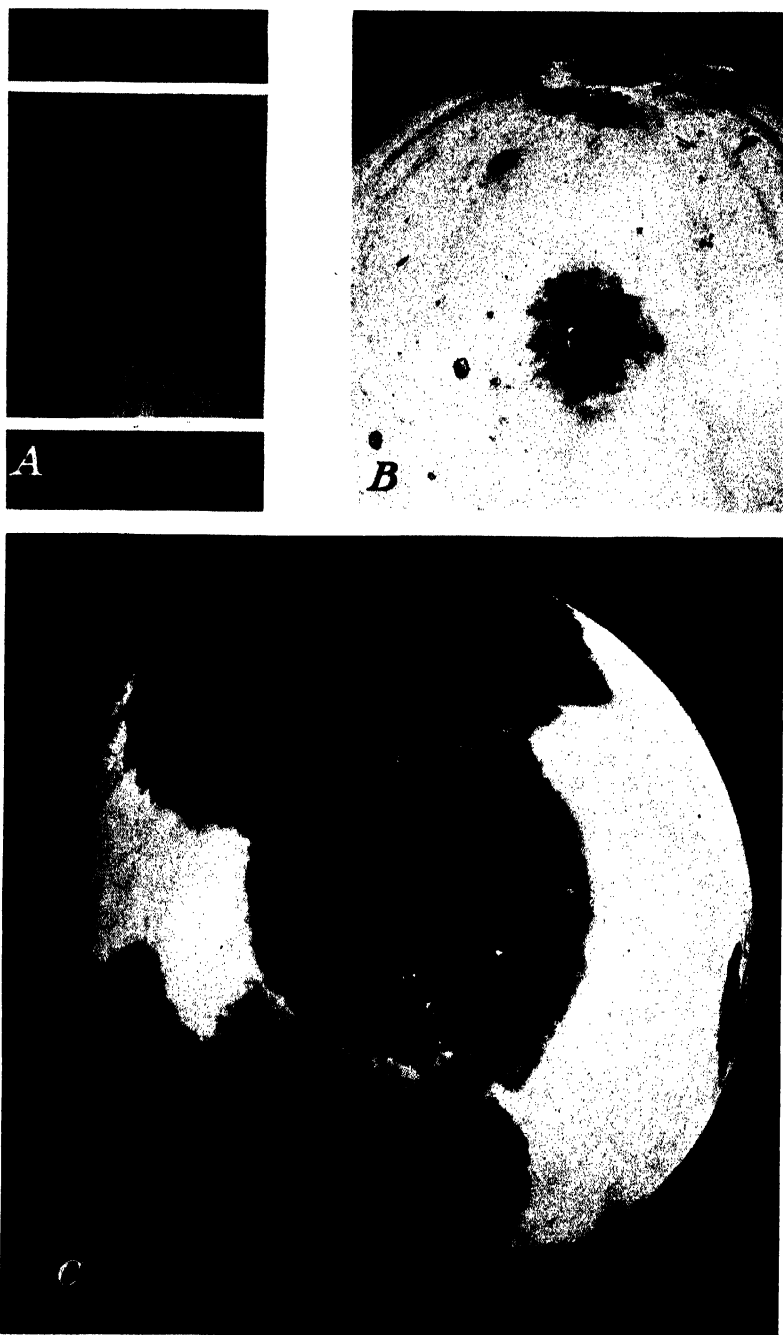


FIGURE 1.—*A*, Early stage of phytophthora rot resulting from inoculation of Winter Queen watermelon; *B*, more advanced stage; *C*, typical lesions on melons from commercial carlot.



FIGURE 2.—Phytophthora rot of Winter Queen watermelon: *A*, Lesion showing mottling, wrinkling, and beginning of mold development; *B*, older lesion nearly covered with mold.

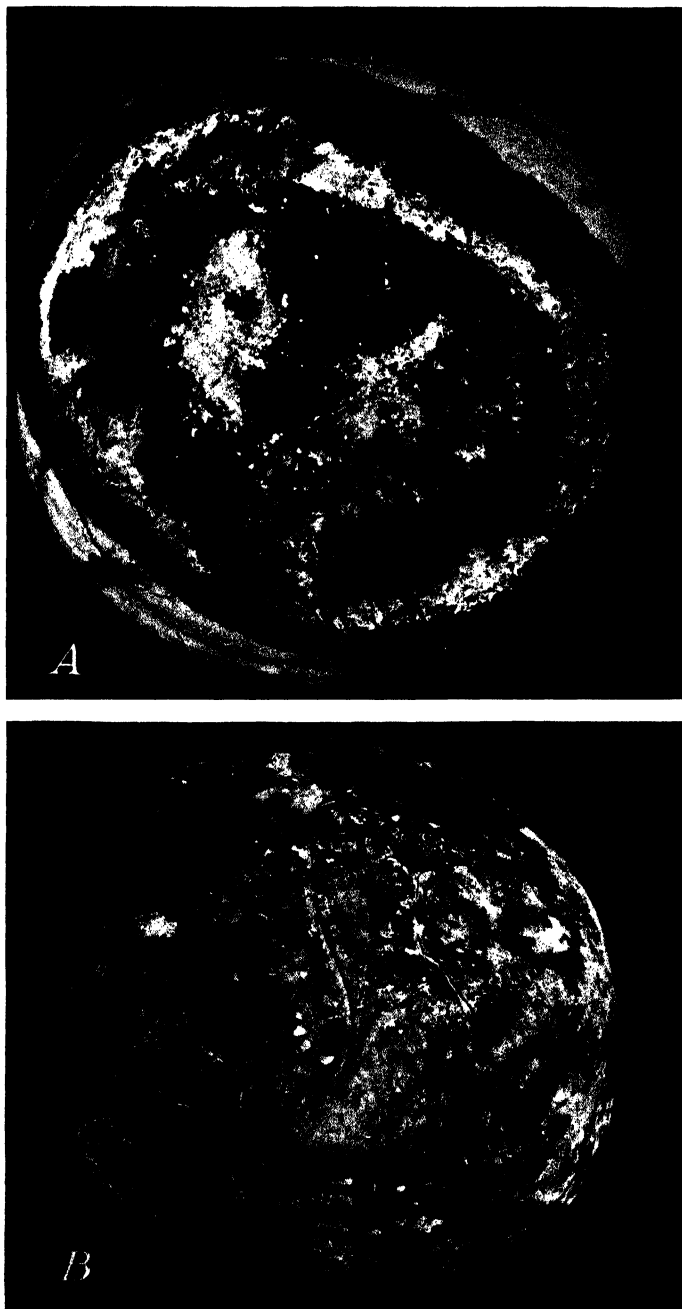


FIGURE 3.—*Phytophthora* rot of Winter Queen watermelon: *A*, Extensive lesion covering nearly half of melon surface; *B*, late stage of decay, entire melon showing shriveling, wrinkling, water soaking, and presence of wet appressed mold.

3, *B*), and the melon either flattens out under the pressure of its own weight (fig. 2, *B*) or otherwise loses its smooth, even, and regular shape (fig. 3, *B*). The epidermis becomes loosened soon after the rind is infected, and can be peeled off readily.

The internal symptoms of decay are a general shriveling and mashing together of the diseased tissues, which remain loosely held together by the fungus mycelium. When the moisture is squeezed out of bits of the affected tissue, the tough, felty mycelium remains as a slight residue. The flavor of healthy tissue somewhat beyond the decayed tissues, unless affected by contaminating yeasts or bacteria, is little affected by the decay. Likewise there occurs no marked odor or discoloration.

THE CAUSAL ORGANISM

MORPHOLOGY AND GROWTH CHARACTERS

Six isolates from rotting Winter Queen watermelon fruits were used for a study of the morphology and growth characters of the fungus. On potato-dextrose agar plates the isolates produced a dense white mat of mycelial growth in the agar, covered with a velvety growth of short aerial hyphae. There was no spore development during a 10-day period. In oatmeal agar tubes there was a mass of white, cottony, aerial mycelium at the base of the slope after 5 days' growth at room temperature. In pea broth, dense tufted masses of mycelium developed throughout the liquid in 6 days, but there was no spore development during that time.

Sporangia developed abundantly in oatmeal and cornmeal agar cultures kept at room temperature for 2 months; they were usually sphaerolimoniform, nonpedicellate, prominently papillate, with granular, hyaline to faintly straw-colored contents, 21.4μ to 53.1μ by 18.3μ to 40.7μ , averaging 36.5μ by 27.6μ in size (fig. 4, *A*). On solid media the sporangia were fairly uniform in size and shape. When the isolates were grown 6 days in pea broth there was a profuse development of mycelium; tufts of hypae washed and transferred to plates of sterile distilled water, as recommended by Leonian (9), developed sporangia in large numbers. They varied widely in size and shape, with a tendency to become elongated in the apical region; the majority had a single papilla, but irregular sporangia with two or three papillae were fairly common. The sporangia produced in water varied from 21.7μ to 60.4μ by 16.9μ to 43.8μ , averaging 40.6μ by 28.3μ in size. Germination of sporangia from both solid and liquid media occurred by the development of germ tubes, usually from the region of the papilla, and by the development and liberation of zoospores, which, after a period of motility, became quiescent, rounded up, and germinated by a single germ tube.

Chlamydospores were not observed.

Three of the six isolates developed oogonia and oospores in oatmeal agar cultures 2 months old. Isolates that developed sexual organs abundantly produced a few sporangia in the same cultures; however, the mycelium of the oogonium-producing isolates developed sporangia as abundantly as the non-oogonium-producing isolates when transferred from pea broth to sterile distilled water but oogonium development did not occur in the distilled water.

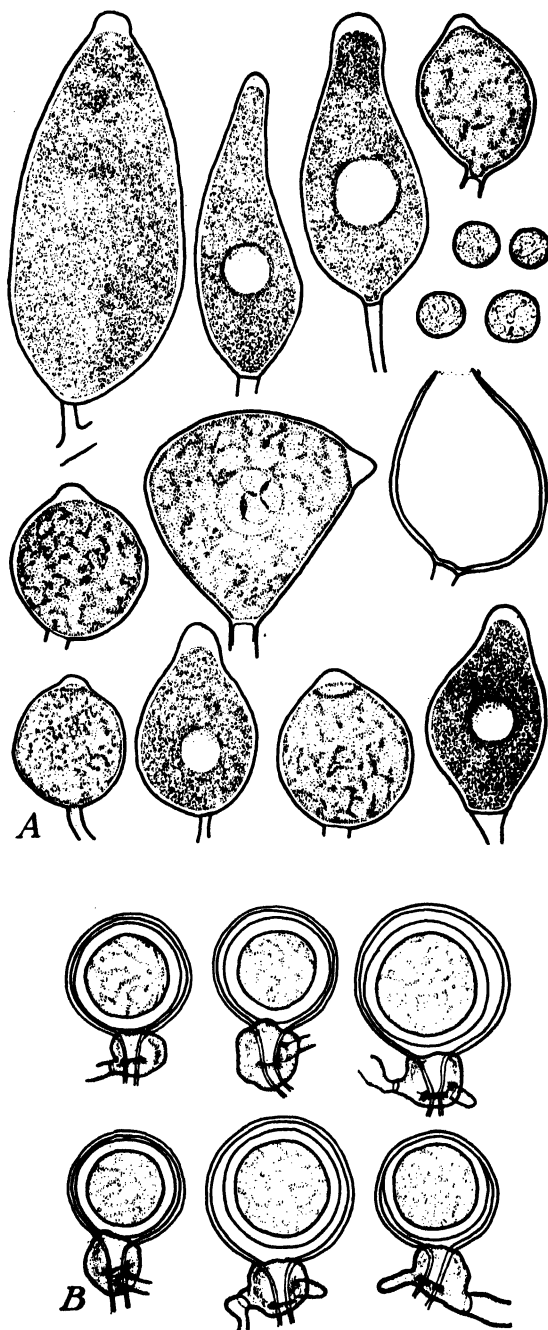


FIGURE 4.—*Phytophthora capsici* from Winter Queen watermelon: A, Sporangia, evacuated sporangium, and rounded, quiescent zoospores; B, oogonia oospores, and antheridia. \times about 600.

The oogonium was subspherical with a slender stalk encompassed by the persistent, amphigynous antheridium. The colorless oogonium wall enclosed dilutely straw-colored granular and often vacuolate contents, which, following fertilization and the differentiation of the oospore, became somewhat darker. The oospores were thick-walled, nearly spherical, not filling the oogonium; a single oospore developed in each oogonium. Oogonia measured 24.1μ to 41.4μ in diameter, averaging 29.9μ . The oospores measured 22.7μ to 31.4μ , with an average diameter of 26.5μ (fig. 4, B).

TAXONOMY

The causal organism was identified as *Phytophthora capsici* Leon. (8). The Winter Queen watermelon isolates were quite similar to strains of that species previously studied by Tucker (17).

Sarejanni (12) in 1936 proposed to combine *Phytophthora capsici* with *P. parasitica*. His proposal would place in the latter species all forms that grow well on malt extract agar and the usual agar media in 6 days at 20°C . and develop amphigynous antheridia and papillate sporangia. He suggested a new species, *P. imperfecta*, for strains with similar growth characters that do not develop oogonia. Strains differing in pathogenicity would be given varietal rank. According to his classification, the three present watermelon isolates that produced oogonia would be assigned to *P. parasitica* var. *capsici* and the three that did not develop oogonia to *P. imperfecta* var. *capsici*.

Conditions influencing the development of sexual organs in cultures of *Phytophthora* species are not well known. Their development is certainly affected by the age of the culture, temperature, substratum, moisture, and the occurrence of heterothallic strains, as well as other undetermined factors. Isolates that produce sexual organs abundantly when first cultured sometimes cease to produce them in later transfers, whereas those failing to produce them in early transfers occasionally develop oogonia and antheridia abundantly in later transfers. The character seems too variable for use as the sole basis of distinction between species of the group with amphigynous antheridia. Several species with nonpapillate sporangia develop oogonia infrequently in culture, yet possess other characters that permit their classification.

Phytophthora capsici is a fairly well defined species, distinguishable from *P. parasitica* by the consistent absence of chlamydospores in culture and by its pathogenicity to pepper plants, a character that has proved stable among isolates from various hosts. The retention of *P. capsici* as a species is therefore highly advisable.

TEMPERATURE RELATIONS

The temperature relations of *Phytophthora capsici* from Winter Queen watermelons were determined by measuring the growth that occurred on plates of potato-dextrose agar⁸ during a period of 4 days at temperatures of 45° to 100°F ., and during 1 week and 2 weeks at 45° , 50° , and 55° . The results are summarized in figure 5, A. The data there shown for the temperatures within the range of 55° to 90° , inclusive, are averages of 4 experiments in each of which 10 plates were measured at each temperature. Thus each point on that

⁸ Pared potatoes, 200 gm.; dextrose, 20 gm.; agar, 15 gm.; distilled water, 1,000 cc.

portion of the temperature-growth curve represents the average diameter attained by the fungus on 40 plates. Growth at each of the temperatures 95° and 100° is based upon the average of 10 plates, while at 45° and 50° it is represented by the average of 35 and of 34 plates, respectively. Six different isolates were used at various times during the 4 experiments.

There was no growth of the fungus at 45° F. in 4 days, 1 week, or 2 weeks. Growth was slight at 50° in 4 days, and moderate in 1 week and 2 weeks. At 55° growth was moderate in 4 days, and extensive in 1 week and 2 weeks.

In later tests slight growth was observed in 1 week at 47.5° F., so that the minimum temperature for growth under the conditions of the test lies somewhere in the range of 45° to 47.5°.

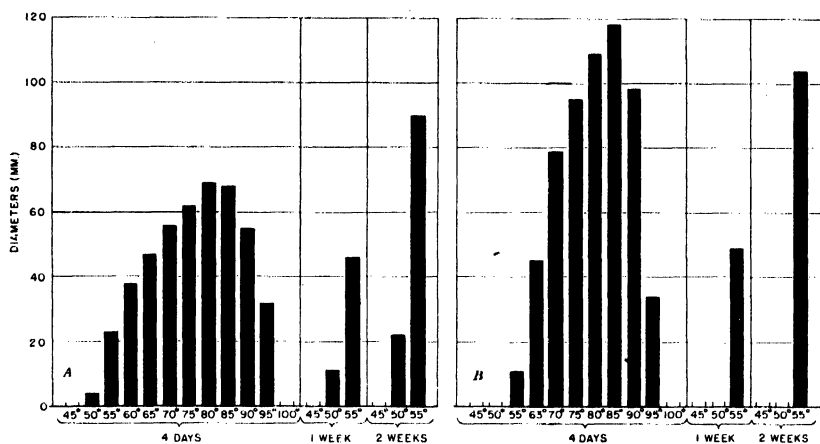


FIGURE 5.—A, Temperature relations of *Phytophthora capsici* from Winter Queen watermelon; average diameter of colonies developed on potato-dextrose agar in 4 days, 1 week, and 2 weeks. B, Temperature relations of phytophthora rot as determined by average diameter of lesions developed on Winter Queen watermelon in 4 days, 1 week, and 2 weeks.

Growth in 4 days increased fairly regularly with increase of temperature from 50° to 80° F., where maximum growth occurred. However, growth was nearly as great at 85° as at 80°. Above 85° growth fell off rapidly with increase of temperature, and no growth occurred at 100° in 4 days. These results are in accordance with data presented by Tompkins and Tucker (15); their isolates from Honey Dew melons grew most rapidly at 25° and 30° C., and all made some growth at 35°.

The effect of temperature upon the rate of development of phytophthora rot was determined by three experiments in which artificially inoculated Winter Queen watermelons were held at various temperatures within the range of 45° to 100° F. The methods used consisted of washing the melons with soap and water, drying them, and placing them in the desired temperature chamber. Twenty-four hours later they were removed, washed thoroughly with 95-percent ethyl alcohol, dried, and inoculated at four points per melon by inserting generous portions of a several-day-old agar culture in shallow wounds made with a sterile scalpel into and through the rind. The

number of melons used varied somewhat, so that the total number of lesions measured in order to determine the average diameter for each temperature point were as follows: At 45°, 40 lesions; at 50°, 42; at 55°, 56; at 63°, 48; at 70°, 36; at 75°, 36; at 80°, 36; at 85°, 36; at 90°, 14; at 95°, 4; at 100°, 8. The results are shown in figure 5, B.

No decay developed at 45° or at 50° F. in 4 days. Some decay occurred at 55° in 4 days; from 55° to 85° the average diameter of the lesions increased regularly with increase in temperature. Maximum decay development occurred at 85°, although the lesions were nearly as large at 80°. Decay development at 90° was about the same as that at 75°, but it fell off materially at 95°, and no decay occurred in 4 days at 100°.

Decay development at 55° F. was moderate in 1 week and advanced in 2 weeks. When the usual method of inoculation was used no decay developed at 50° even in 2 weeks. However, when melons with definite lesions of phytophthora rot were held at 50° for 1 week and 2 weeks the lesions continued to enlarge moderately. In no case was there any decay development at 45°.

PENETRATION STUDIES

Observations made on commercial lots of Winter Queen watermelons showed that in many crates phytophthora rot was more abundant at points of contact between adjacent melons than elsewhere over the melon surface. This suggested the possibility of spread from diseased to healthy melons within the container. A number of small-scale experiments were therefore made for the purpose of determining whether the pathogen could penetrate the apparently unbroken skin of the melon and whether or not the decay would spread from melon to melon under conditions simulating those existing in commercial practice.

Melons selected for their general freedom from blemishes and scratches were washed with soap and water, dried, washed with 95-percent ethyl alcohol, and again allowed to dry. Small plantings of potato-dextrose agar cultures of the fungus previously isolated from decaying Winter Queen watermelon were then placed at isolated spots on the melon surface where the rind appeared to be entirely free of blemishes or injuries of any sort. These were covered with inverted sterilized wide-mouth vials,⁹ which were fastened to the melon by means of melted paraffin wax. In some instances a piece of sterilized cotton moistened with sterilized water was laid over the agar-mycelium planting before the vial was sealed on. In others no cotton was used, but the inside of the vial was moistened with sterile water. Suitable checks, consisting of sterile agar plantings, were similarly set up.

In 1 such test 3 melons were each inoculated at 10 points with 1 of 3 different isolates. Three days later 90 percent of infection had occurred with each of 2 isolates, and 70 percent with the third isolate. No decay developed on the checks.

A second test consisted of 19 inoculations, of which 17, or 89 percent, resulted in infections with phytophthora rot.

In a final test of a similar type, in which moistureproof cellophane firmly secured around the edges was substituted for the glass vials, all but 1 out of a total of 15 inoculations were positive within 6 days.

⁹ Lipless thick-walled glass bottles 25 mm. in diameter and 70 mm. in length, of the type known as shell vials.

Evidences of infection were visible within 2 or 3 days at room temperature.

The results of the three tests indicate that *Phytophthora capsici* is capable of penetrating the rind of Winter Queen watermelons in the absence of visible scratches or injuries in the rind.

In the first test made to determine whether spread of decay occurs within the crate, 5 healthy melons and 3 melons bearing advanced lesions of phytophthora rot were laid together within a large basket in such a manner that 15 points of contact were made between healthy and diseased rind. The healthy melons bore no fresh bruises nor wounds. They were not washed, nor were their surfaces sterilized. All were held at a mean daily temperature of about 70° to 75° F. for 4 days. At the end of that time phytophthora rot was well advanced on the previously healthy rind at each of the 15 places where contact had been made with infected melons. At the start of the test the decay lesions on the 3 infected melons were more moist than the earlier stages of decay as ordinarily found in commercial packages. Moreover, all 8 melons were packed together somewhat more tightly than in commercial packing.

Three tests were therefore made, in each of which only early to moderately advanced stages of decay were used as the source of inoculum and in which the diseased and healthy melons were packed in crates with pressure of melon against melon approximating that found in commercial crates. In all 3 tests the healthy melons were washed and dried, and the areas of healthy rind exposed to the decay lesions were selected for freedom from fresh scratches and wounds. The arrangement was such that the diseased melons made 10 contacts with 8 sound melons. The crates were held at room temperatures approximating a daily mean of 72° F. for 6 days. Phytophthora rot had by that time spread to the healthy melons at all 10 points of contact. Similarly, in a repetition of this test with other melons, there was infection at all 10 points of contact in a 4-day period. In a final test of this sort 20 contacts were made between healthy and diseased melons. After 5 days phytophthora rot had spread to the healthy melons at 18 points.

The four tests show clearly that phytophthora rot can spread from melon to melon within the container, and that by the end of 4 days at temperatures near 70° F. lesions are developed that are of considerable economic importance.

HOST RANGE

Inoculations with the six isolates of *Phytophthora capsici* were made by the method used by Tompkins and Tucker (15). All isolates were found to be similar in pathogenicity and proved to be capable of penetrating unwounded tissues and causing rots of the fruits of pepper, tomato, apple, cantaloup, cucumber, and squash, and of carrot roots. They infected wounded fruits of orange and lemon, and wounded potato tubers. The symptoms produced on the various commodities agreed very closely with those reported by one of the writers (17), using isolates from pepper, and by Tompkins and Tucker (15), using isolates from Honey Dew melons.

Inoculations of turnip and radish roots and of onion bulbs were negative.

The pathogenicity of *Phytophthora capsici* for pepper plants has been used as a criterion for the identification of the species (17). California Wonder pepper plants were therefore inoculated with the six watermelon isolates by placing a bit of mycelium from oatmeal-agar cultures in a small slit in the stem near the tip. Twenty-four plants were thus inoculated, each isolate on four plants. All isolates proved virulently pathogenic, killing the terminal growing region and causing browning and collapse of the stems 4 to 6 inches below the point of inoculation within 6 days (fig. 6). Inoculations of young pepper plants, made by mixing cultures with the soil, caused wilting and death of all plants within 25 days. The watermelon isolates were as pathogenic for pepper plants as were isolates from pepper and Honey Dew melon.



FIGURE 6.—California Wonder pepper plants: A, Wounded but not inoculated; B, inoculated with *Phytophthora capsici* isolated from Winter Queen watermelon. Photographed 6 days after inoculation.

CONTROL

As has been pointed out, the organism that causes the phytophthora rot of Winter Queen watermelons has been likewise responsible for a field decay of Honey Dew melons in California. There the disease was found restricted to areas of irrigated fields in which excessive water accumulated as a result of poor drainage. In Colorado the same fungus was reported (1) on peppers in the vicinity of Rocky Ford in 1931 and at Canon City in 1932. The disease was most prevalent in heavy wet soils. What is apparently the same fungus was also reported (7) as the cause of a field decay of cucumber fruits at Rocky Ford in 1936.

The causal fungus is soil-borne and has been shown (1) to be able to remain alive in the soil of coldframes for at least 2 years. It would therefore seem advisable to avoid planting Winter Queen watermelons in fields where infections by *Phytophthora capsici* have recently occurred on pepper, cucumber, or other crops.

Although no information is available on the occurrence of the disease in the fields of Colorado, where the infected Winter Queen watermelons originated, the wide variations in the percentage of melons affected in different shipments and during different seasons suggest that environmental factors in the field play an important role in determining the importance of the disease during marketing. The presence of excessive soil moisture is undoubtedly an important factor, and where this condition prevails it would be well to determine, prior to the time of picking, whether the decay is present on fruits in the field. If so, then care may be exercised in harvesting to avoid shipping melons from such areas in the field. However, should the fruit rot be found rather generally distributed, even in small amount, over the field, it should be recognized that the chances for serious development of decay during the transit and marketing period are greatly increased.

The prevalence of unusually wet conditions shortly before harvest may result in extensive infection in fields where the pathogen is present and active. Melons that have been only recently infected may show no evidence of the disease at the time of packing. Under temperatures prevailing in cars shipped under ventilation without ice, such melons soon become extensively rotted and may readily serve as centers for spread of the decay to adjacent melons in the containers. From the results of the temperature studies it would appear that reduction of carrying temperatures to 50° F. through the use of refrigeration would materially retard decay and that maintenance of a temperature of 45° would completely arrest the development and spread of the decay during the transit period.

SUMMARY

A phytophthora rot of Winter Queen watermelons grown in Colorado was first observed on the New York City market in 1935.

During the four-season period of observation it was found that the disease occurred sporadically, but that when present in a given carlot it might affect from 5 percent or less to nearly all of the melons.

The causal organism was found to be *Phytophthora capsici* Leon.

The optimum temperature for growth of the pathogen in culture was in the range of 80° to 85° F. The maximum temperature (for growth in 4 days) lay between 95° and 100°. The minimum temperature for growth of the organism lay between 45° and 47.5°. Growth did not occur at 45° in 19 days.

The development of decay on inoculated Winter Queen watermelons was greatest at 85° F. At temperatures in the range of 63° to 70°, lesions attained a diameter of 45 to 79 mm. (approximately 2½ to 3 inches) in 4 days. At 50° old lesions continued to enlarge slowly, but new lesions were not apparent in 14 days. No decay developed at 45° in 14 days.

The causal fungus was found to be able to penetrate the apparently unbroken skin of Winter Queen watermelons. Tests indicated that at favorable temperatures the decay can readily spread within the container from diseased to healthy melons.

Tests showed that the causal fungus was pathogenic for pepper plants and that it could penetrate and rot unwounded fruits of pepper, tomato, apple, cantaloup, cucumber, and squash, and unwounded

carrot roots. The fungus also produced a rot of wounded orange and lemon fruits and wounded potato tubers.

Suggestions for control, based upon certain field and harvesting practices, are presented. Reduction of transit temperatures to 50° F. should greatly retard decay development, and the maintenance of a temperature of 45° should completely arrest the development and spread of decay during transit.

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THE USE OF ASCORBIC ACID (CRYSTALLINE VITAMIN C) AS A SUBSTRATE IN OXIDASE MEASUREMENTS¹

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INTRODUCTION

During storage the physiological changes in fruits are katabolic, and oxidative processes are doubtless dominant. A study of these processes is of importance from the standpoint of fruit behavior and particularly with reference to physiological diseases such as soft scald and break-down of apples and core break-down of pears. A few degrees difference in storage temperature may mean either severe losses or fruit comparatively free from disease. Thus, at 30° or 32° F. certain varieties of apples may develop severe soft scald, whereas at 36° this disease very seldom develops. However, at the higher temperature the period of profitable storage is shortened owing to more rapid ripening. These results indicated that a study of the oxidizing enzymes might be of considerable importance for an understanding of the basic causes of this type of disease and in turn might lead to methods for its control. Preliminary tests indicated that the difference in oxidase activity of fruit stored at 32° and 36° might be small. A more sensitive method than had previously been available for measuring small changes in enzyme activity was needed, and the present study was therefore undertaken.

Oxidase activity may be determined colorimetrically (16, p. 234),² volumetrically (4), or iodometrically (3). Of these three processes, the last is in many respects the most satisfactory. In Guthrie's (3) iodometric method the oxidizable substrate is prepared by heating glucose with a dilute sodium hydroxide solution. The glucose is broken down into numerous derivatives, some or all of which reduce iodine in acid solution. These carbohydrate degradation products are unstable and lose their oxidizing power in the presence of air, and their oxidation is catalyzed by plant juices, i. e., by oxidase.

When glucose is heated with dilute alkali, a mixture of various decomposition products is formed. It is conceivable that some of these carbohydrate derivatives may require different degrees of oxidation before their iodine-reducing power is destroyed, while other products in this mixture may possess no iodine-reducing power or may even have an inhibitory action on the oxidase enzyme. For this reason it would seem desirable to have a pure substrate in which the oxidizable material would be uniform and other products would not be present as a possible source of interference with the enzyme action.

In the quantitative determination of ascorbic acid in plant tissues it is necessary to inactivate the plant oxidase to prevent destruction of the acid. Szent-Györgyi (13) noted the reducing power of plant juices and identified the reducing substance as hexuronic acid. This

¹ Received for publication April 1, 1939.

² Italic numbers in parentheses refer to Literature Cited, p. 98.

was later termed ascorbic acid and is now available in crystalline form. Ascorbic acid, then, is a definite chemical compound; it may be obtained in pure form; it is readily oxidized by the oxidase enzyme; and the amount of oxidation may be determined by iodine reduction in acid solution. It therefore appeared likely, and preliminary tests indicated, not only that ascorbic acid could be used as a substrate for oxidase measurements but that it possessed distinct advantages over the glucose-derivative substrate as prepared by Guthrie.

This paper presents the results of a study in which ascorbic acid (crystalline vitamin C) is used as a substrate for oxidase measurements in comparison with the glucose-derivative substrate of Guthrie. The influence of concentration of the enzyme, of the ascorbic acid, of the acidity, and of various other factors has received attention.

MATERIALS AND METHODS

The enzyme preparation consisted of the pressed juice in most cases. The tissue to be used was ground in a food chopper with a nut-butter attachment, and the juice was squeezed through two thicknesses of cheesecloth. The juice was then filtered through double cheesecloth, without pressure, to remove any tissue forced through at the first filtering.

The method of measuring oxidase activity was similar to that described by Guthrie (3) except that 25 cc. of a water solution of ascorbic acid crystals (cevitamic acid, Merck) was used, usually in concentrations of 0.1 to 0.4 percent, as a substrate in the Van Slyke-Cullen aeration tubes, instead of the heated glucose solution of Guthrie. From 1 to 10 cc. of the juice or extract containing the enzyme was added to each tube, the amount depending on the oxidase content. The boiled preparation was used as a blank. Whenever the enzyme-substrate solution foamed badly, 5 drops of paraffin oil were added to each tube. Uniform reaction conditions were obtained by drawing air for 1 hour, at the rate of 8 liters per hour, through a constant-temperature bath held at 25° C. After aeration the solutions were transferred with 50 cc. of water to 500-cc. Erlenmeyer flasks containing 25 cc. of 10-percent trichloroacetic acid. Twenty cubic centimeters or more of N/50 iodine in N/10 potassium iodide was then added, and after standing for 30 minutes the solution was titrated with N/100 sodium thiosulfate. The difference in titration between the boiled and the unboiled sample gave a measure of the oxidase activity. When several samples are aerated simultaneously, care should be taken to keep the iodine oxidation-reduction period uniform.

EXPERIMENTAL WORK

EFFECT OF THE HYDROGEN-ION CONCENTRATION OF THE SUBSTRATE ON OXIDASE ACTIVITY

Water solutions of ascorbic acid are acid in reaction. Falk (2, p. 98) states that the optimum pH value for vegetable oxidase activity is from 7 to 10. This is more alkaline than most plant juices. Apple juice has a pH value of approximately 4. With potato juice and a sugar-derivative substrate, Guthrie (3) suggests a pH value of 6.5 for oxidase measurement.

In order to determine whether the acidity of the ascorbic acid solution interfered seriously with the oxidase activity, this substrate

was adjusted to various acidities with N/10 sodium hydroxide and buffered with Clark and Lubs buffer solutions. Equal parts of the substrate and of buffer solutions were used and adjusted to a constant volume and concentration. The oxidase activity of apple juice was then determined at various acidities. The results are given in table 1, column 2.

The substrate with a pH value of 4.0 was more acid than is usually recommended for optimum oxidase activity, but the use of sodium hydroxide and buffer solutions to give a pH value as high as 8.0 did not increase the enzyme activity. The fact that there was such a wide difference between the check (unbuffered substrate) and the sample buffered to pH 4.0, both of which were near the same acidity, suggested that the buffer chemicals were interfering with the enzyme action. This proved to be true, as is shown in table 1, column 3, when the substrate was brought to the desired pH value with N/10 sodium hydroxide without addition of buffer. With the acidity thus reduced enzyme activity was again less than in the unneutralized substrate.

TABLE 1.—Effect of the hydrogen-ion concentration of the substrate on oxidase activity

Ascorbic acid substrate (pH) ¹	Oxidase activity (N/100 Na ₂ S ₂ O ₄)		
	Apple juice (substrate buffered)	Apple juice (substrate unbuffered)	Potato juice (substrate unbuffered)
(1)	(2)	(3)	(4)
Check ²	Cc.	Cc.	Cc.
4.0	6.2	8.5	7.1
5.0	7	8.5	7.1
6.0	2.6	6.6	6.5
7.0	3.8	6.1	7.3
8.0	2.7	5.2	7.1
8.0	1.5	5.2	7.2

¹ N/10 NaOH added to secure pH value desired.

² 0.1-percent water solution, pH value approximately 4.0

Since apple juice has a pH value approximately equal to a 0.1-percent solution of ascorbic acid, it was thought that an enzyme preparation less acid than apple juice might behave differently in regard to the acidity of the substrate. Although repeated tests with potato juice having a pH value of 6.2 did not always show increased oxidase activity with the more alkaline substrates, the effect of adjustment did not appear to be harmful. The results of one of these tests are given in table 1, column 4. These results with apple and potato juice might indicate that the optimum pH value for oxidase activity is near that of the original material; i. e., apple near pH 4.0 and potato near pH 6.2. The failure of the potato juice to show definitely greater activity with the adjusted substrates may be due to the injurious effect of the sodium hydroxide on the enzyme, offsetting the advantages of increase in alkalinity.

EFFECT OF CONCENTRATION OF SUBSTRATE ON OXIDASE ACTIVITY

The effect of concentration of the substrate on the oxidase activity of apple juice is shown (table 2) under conditions in which the concentration of the ascorbic acid ranged from 0.1 to 0.4 percent. Two cubic centimeters of apple juice was used at each concentration.

Differences in concentration of the substrate from 0.1 to 0.3 percent of ascorbic acid had little effect on the enzyme activity, whereas 0.4 percent was strong enough to cause injury to the enzyme (table 2, column 2). Guthrie (3) has stated that boiled juice has a protective effect on the oxidase enzyme. When 8 cc. of boiled juice was added to 2 cc. of unboiled juice and the test repeated, no injury at 0.4 percent was evident (table 2, column 3). In a later test, with onion juice very weak in oxidase, a 0.3-percent ascorbic acid solution showed slight injury to the enzyme.

TABLE 2.—Effect of concentration of substrate on the oxidase activity

Concentration of substrate (percent)	Oxidase activity (N/100 $\text{Na}_2\text{S}_2\text{O}_3$)	
	Enzyme unprotected	Enzyme protected ¹
(1)	(2)	(3)
	Cc.	Cc.
0.1.....	9.9	12.8
0.2.....	9.9	13.0
0.3.....	9.9	13.0
0.4.....	8.0	13.0

¹ Enzyme protected by the addition of 8 cc. of boiled juice to 2 cc. of unboiled juice.

For routine analysis, where the titration difference between the boiled and the unboiled juice is not more than 15 cc. of N/100 thiosulfate, a substrate concentration of 0.1 percent of ascorbic acid has been found satisfactory. Since 1 cc. of N/100 thiosulfate is equivalent to 0.88 mg. of ascorbic acid (8), a titration of 15 cc. would indicate that 13.20 mg. of ascorbic acid had been oxidized by the enzyme. The amount oxidized by the blank varies with the product and with the amount of boiled material used: 2 cc. of boiled apple juice oxidized

1.85 mg. of ascorbic acid during aeration; a difference of 15 cc. in titration would indicate that approximately 60 percent of the substrate had been oxidized.

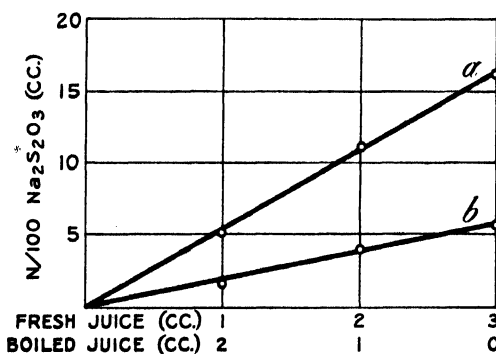


FIGURE 1.—Effect of concentration of the enzyme on the oxidase activity of apple juice: a, Relatively strong oxidase solution; b, relatively weak oxidase solution

EFFECT OF CONCENTRATION OF ENZYME ON OXIDASE ACTIVITY

When the protective action of the juice is kept constant by the addition of boiled juice, the oxidase activity varies directly with the concentration of the enzyme.

This is shown in figure 1, where the activity of apple juice relatively strong and weak in oxidase is plotted.

EFFECT OF THE SUBSTRATE AND ITS OXIDATION PRODUCTS ON THE ENZYME

The product of a reaction often interferes with the action of the enzyme, and as a result the concentration of active enzyme may change during the course of the reaction. For instance, when 2-cc. samples of Winesap apple juice were aerated for 20, 40, and 60 minutes, the oxidase activity was 3.4, 6.7, and 9.7 cc., or 3.4, 3.3, and 3.0 cc. for the three 20-minute intervals, respectively. This reduction in enzyme activity may be due to the action of the substrate or to the products formed by the reaction between the enzyme and substrate. Guthrie (3) noted that this destructive action was reduced by the addition of boiled juice to the unboiled juice. To determine whether the ascorbic acid substrate was as injurious to the enzyme as the glucose-derivative substrate, a test was made in which both ascorbic acid and glucose-derivative substrate were used. In each case the quantity of unboiled juice was kept constant while the quantity of boiled juice was varied. The results are shown in table 3.

It is evident that the boiled juice was more effective in increasing oxidase activity with the heated glucose-derivative substrate than with the ascorbic acid substrate. Also the difference between 2 and 4 cc. of boiled juice was much greater in the glucose-derivative substrate than in the ascorbic acid substrate. Conversely, it may be stated that the heated glucose-derivative substrate or the products of its oxidation are more destructive to the enzyme than is the ascorbic acid substrate or its oxidation products. The substrate, reduced or oxidized, should be as free as possible from such destructive substances.

TABLE 3.—*Effect of different substrates on the oxidase activity of apple juice when various proportions of protective boiled juice are used*

Enzyme preparation	Activity of apple juice on—			
	Ascorbic acid substrate		Glucose-derivative substrate	
	Oxidase activity (N/100 Na ₂ S ₂ O ₃)	Increase over unprotected juice	Oxidase activity (N/100 Na ₂ S ₂ O ₃)	Increase over unprotected juice
	Cc.	Percent	Cc.	Percent
2 cc. of unboiled juice, no boiled juice	7.0		4.2	
2 cc. of unboiled juice + 2 cc. of boiled juice ..	9.4	34.3	6.3	50.0
2 cc. of unboiled juice + 4 cc. of boiled juice	10.0	42.8	7.8	85.7

Johnson and Zilva (5) have shown that certain plant enzymes oxidize catechol in catechol-ascorbic acid mixtures but only after the ascorbic acid is all oxidized. This would indicate that ascorbic acid is more readily oxidized than catechol. If increasing amounts of an enzyme preparation are added to a constant amount of ascorbic acid substrate until an excess of enzyme is present, a slight excess of enzyme shows no increase in oxidase activity and no difference in the color of the solution. However, if an additional excess is added, further visible oxidation takes place and darkening results, the amount of darkening depending roughly on the excess enzyme added. It should be noted that visible darkening does not take place until all of the ascorbic acid is oxidized. According to Onslow (10, p. 122) oxidase

is made up of three components, one of which is an aromatic substance containing an orthodihydroxy grouping such as that in catechol. The darkening may be due to the oxidation of this aromatic compound. This would seem to agree with Johnson and Zilva's results, in which the catechol, in ascorbic acid-catechol mixtures, was not oxidized until all of the ascorbic acid had been oxidized.

The direction of the ascorbic acid oxidation appears somewhat obscure. Ascorbic acid is generally considered to be first oxidized to dehydroascorbic acid, which is approximately equal to ascorbic acid in antiscorbutic potency. According to Borsook et al. (1), dehydroascorbic acid in aqueous solutions undergoes a spontaneous irreversible change at ordinary temperatures if the hydrogen-ion concentration is less than pH 4, forming a stronger acid and a more powerful reducing agent than ascorbic acid itself. However, this change is independent of the presence of air or oxidizing agents; consequently it is not an oxidation. Moll and Wieters (9) found that pure aqueous solutions of dehydroascorbic acid are far less stable than ascorbic acid solutions, and that the antiscorbutic property is lost more quickly than the property of being regenerated by hydrogen sulfide. This degradation product is an ascorbic-acid-like substance with a characteristic reductive action on iodine, silver nitrate, and dichlorophenol-indophenol, but without antiscorbutic properties. In order to determine whether the products of the ascorbic acid oxidation were reducing the iodine in a similar manner to the unoxidized ascorbic acid the following study was made, in which the enzyme was permitted to act on the substrate over a long period of time and in which the oxidation products were allowed to stand in strongly acidified solutions. The results are as follows:

Lot No. and treatment:	Oxidase activity (cc. N/100 Na ₂ S ₂ O ₃)
(1) Check, 2 cc. of enzyme material, aerated 1 hour; oxidase determined by usual procedure.....	2. 9
(2) 2 cc. of enzyme material, aerated 1 hour and then let stand for 17 hours at 27° C. before adding acid and iodine and determining the activity.....	4. 6
(3) 2 cc. of enzyme material, aerated 1 hour; then 25 cc. of 10-percent trichloroacetic acid added and let stand 17 hours at 27° C. before iodine added and activity determined.....	3. 0
(4) 10 cc. of enzyme material, aerated 1 hour, but let stand 17 hours at 27° C. before acid and iodine added and activity determined.....	1 23. 6
(5) 10 cc. of enzyme material, aerated 1 hour; then 25 cc. of 10-percent trichloroacetic acid added and let stand 17 hours at 27° C. before iodine added and activity determined.....	1 20. 0

¹ All of the substrate was oxidized in lots 4 and 5. The apparent difference in activity was due to difference in the blanks. The blank in lot 5, acidified following aeration, was greater than the blank in lot 4. The thiosulfate titration was practically the same in all tubes containing the enzyme in the 2 lots.

From the above data, it appears that the degradation products did not react with the iodine as the ascorbic acid did even when the enzyme was allowed to act for 18 hours or when the oxidized substrate was allowed to stand in a strongly acidified solution for 17 hours. Lot 1 was a comparatively weak enzyme solution and oxidized but little of the substrate when determinations were made by the usual procedure. Lot 2, in which the enzyme was allowed to act without aeration for an additional 17 hours, showed 58 percent more of the substrate oxidized than lot 1. Lot 3 had the enzyme killed at the end of aeration and, as would be expected if the oxidized substrate

did not react with the iodine, showed practically the same activity as lot 1. Lots 4 and 5, containing 10 cc. of the enzyme, oxidized all the substrate in less than 1 hour as was shown by a brownish discoloration at the end of the aeration. Lot 4 showed increased darkening after 18 hours and was distinctly darker at the end of the period than lot 5, in which the enzyme was killed at the end of aeration. Yet no lot gave any indication that the oxidized substrate was reducing the iodine as occurs in the case of ascorbic acid. The apparent difference in activity between lots 4 and 5 is due to a difference in the blanks; the acidified blank gave a higher thiosulfate titration than did the one that remained unacidified overnight. It should be recalled that the thiosulfate titration is a measure of the excess iodine added after the enzymic oxidation of the ascorbic acid; it is directly proportional to the enzyme activity and inversely proportional to the ascorbic acid remaining in the substrate.

Even though the reducing substance formed from dehydroascorbic acid is present in the oxidized substrate, it still would be ineffective in reducing the iodine. Borsook et al. (1) have shown that it is inactive as a reducing agent below pH 4. The acidified substrate, containing 25 cc. of 10-percent trichloroacetic acid to which the iodine is added, is considerably lower than pH 4. At no time has there been any indication of a reducing substance being formed in the oxidized substrate.

ENZYMIC VERSUS METALLIC OXIDATION OF THE ASCORBIC ACID

It is well known that copper can catalyze the oxidation of ascorbic acid. Stotz et al. (12) found that the copper in the so-called "ascorbic acid oxidase" from squash and cauliflower was sufficient to account for the oxidation credited to the enzyme and that the greater part of the copper could be recovered in the coagulated protein upon heat inactivation. In the present work practically the same results were obtained whether the enzyme was killed by heating or by the addition of 10-percent crystalline trichloroacetic acid to the juice. Other metals also may catalyze the oxidation of ascorbic acid (7). That grinding the tissue through the food chopper had no effect on the rate of oxidation of the ascorbic acid was shown by using ripe peaches in which the juice could be hand-pressed without cutting or grinding. Hand-pressed juice gave the same activity as did the juice from similar tissue ground in the food chopper before pressing.

That the oxidation of the ascorbic acid was due to an oxidizing enzyme and not to copper or other metals present in the juice is shown by the results of an experiment (table 4) in which the oxidase activity of the same enzyme preparations was determined at various intervals of time. The enzyme solutions were held in stoppered Erlenmeyer flasks at room temperature throughout the test. If the oxidation were due to copper, then with the copper content of the juice remaining constant the rate of oxidation of the substrate should also remain constant; but if the oxidation were due to an enzyme, the enzyme gradually would be destroyed with standing, and the rate of oxidation should decrease. The data in table 4 show that the ability to oxidize the substrate diminishes with time. This would indicate that the oxidation is enzymic in character.

Other indications that the oxidation is due to an enzyme and not to copper that might be present in the juice are given in table 3 and

on page 93. The addition of boiled juice (table 3) would not be expected to exert a protective action on the copper but might be expected to do so with enzymes. Also, the copper content being constant, the amount of oxidation would be expected to remain constant during equal intervals of time. That such is not the case is shown on page 93.

COMPARATIVE EFFECT OF DIFFERENT SUBSTRATES ON THE OXIDASE ACTIVITY OF VARIOUS PLANT PRODUCTS

The relative oxidase activity of various plant products was determined with ascorbic acid substrate and with the glucose-derivative substrate prepared as recommended by Guthrie (3). The results are given in table 5.

TABLE 4.—*Effect of time on oxidase activity*

Time elapsed after preparation	Oxidase activity (N/100 $\text{Na}_2\text{S}_2\text{O}_3$) of—	
	Delicious apple juice	Golden Delicious apple juice
	Cc.	Cc.
2 hours.....	9.5	8.0
26 hours.....	5.7	6.2
45 hours.....	2.1	3.8
15 days ¹	0	.5

¹ Slight growth of mold in solutions at this time.

TABLE 5.—*Oxidase activity of various plant products as influenced by the kind of substrate*

Source of enzyme		Oxidase activity (N/100 $\text{Na}_2\text{S}_2\text{O}_3$)		Source of enzyme		Oxidase activity (N/100 $\text{Na}_2\text{S}_2\text{O}_3$)	
Material	Quantity	Ascorbic acid substrate	Glucose-derivative substrate	Material	Quantity	Ascorbic acid substrate	Glucose-derivative substrate
	Cc.	Cc.	Cc.		Cc.	Cc.	Cc.
Winesap apple juice (fruit).....	2	7.1	3.9	Cantaloupe juice (melon).....	10	0.7	0.0
Red Rome apple juice (fruit).....	5	8.1	5.5	Lettuce juice (head).....	2	6.2	2.0
Rose pear juice (fruit).....	2	2.3	1.0	Cucumber juice (fruit).....	2	26.1	15.5
Bartlett pear juice (fruit).....	2	4.0	1.7	Watermelon juice (red meat).....	10	0	0
Peach juice (fruit).....	5	12.8	5.7	Orange juice (fruit).....	10	0	0
Plum juice (fruit).....	5	15.4	7.7				
Potato juice (tuber).....	2	24.6	15.5	Average.....		6.52	3.28
Onion juice (bulb).....	5	1.4	1.1				
Carrot juice (root).....	5	8.3	1.3	Average increase over glucose-derivative substrate.....		Percent 98.7	
Radish juice (root).....	5	0	0				
Cabbage juice (head).....	2	1.6	.8				
Water suspension of—							
Dried cherry bark.....	2	1.2	0				
Do.....	4	2.0	.4				
Fresh cherry bark.....	5	2.4	.3				

It is interesting to note that the average oxidase activity of these various plant products was 98.7 percent greater with the ascorbic acid substrate than with the sugar-derivative substrate. This greater activity is especially important where the amount of enzyme activity is small. In some cases where no activity was evident with the glu-

cose-derivative substrate, definite activity was shown with the ascorbic acid substrate. That the ratio of activity for the substrates did not remain constant with different enzyme preparations was probably due to the varying amounts of the injurious substances in the sugar-derivative substrate or to the relative immunity of the various preparations to injury from such substances. Table 3 shows that the sugar-derivative substrate was more injurious to the enzyme than was the ascorbic acid substrate.

DISCUSSION

In the determination of ascorbic acid in plant tissues by dichlorophenolindophenol or other chemical means it is often necessary to inactivate the plant oxidase to prevent the ascorbic acid from being oxidized to dehydroascorbic acid, which is biologically active but does not react with the dichlorophenolindophenol dye. Plants differ considerably in the rate at which this change takes place. Stone (11) concluded that it was a question of ascorbic acid oxidase. If the oxidase was present the plant lost the indophenol-reducing power on mincing; if the oxidase was absent the indophenol-reducing power remained.

Several workers (5, 6, 11, 13, 14, 15) have reported the presence of an ascorbic acid oxidase that can oxidize ascorbic acid. Szent-Györgyi (13), the first to note this property, states that the hexuronic acid (ascorbic acid) oxidase is not a single enzyme but apparently involves a number of catalysts. Tauber et al. (15) isolated an ascorbic acid enzyme from the pericarp of Hubbard squash which gave none of the color reactions that other oxidases give with such reagents as benzdine, guaiacol, pyrogallol, catechol, phloroglucinol, resorcinol, naphthoresorcinol, or vanillin. Nor did it affect glutathione, cysteine, tyrosine, adrenalin, or glucose boiled with alkali, and its kinetics indicated a single enzyme. However, they found practically no ascorbic acid, reduced or oxidized, in the fruit, so the role of this enzyme in the physiology of the plant remained obscure.

Copper catalyzes the oxidation of ascorbic acid very readily, and precautions must be taken to prevent it from oxidizing the ascorbic acid in the dichlorophenolindophenol method of analysis (7). Stotz et al. (12) studied the role of copper in the ascorbic acid oxidase from squash and cauliflower and reported that the copper content was sufficient to account for the observed catalysis and that the greater part of the copper in such preparations was found in the coagulated protein upon heat inactivation. Additional results strengthened the indication that copper was responsible for the oxidation of the ascorbic acid. Stotz et al. (12) denied any need for using such terms as "ascorbic acid oxidase," "vitamin C oxidase," or "hexuronic acid oxidase," at least in the sense that they have been used in the past.

However, it is generally recognized, and the work reported herein emphasizes, that there are certain plant enzymes that are capable of oxidizing ascorbic acid. These same enzyme preparations oxidize the degradation products formed when glucose is heated with an alkali, and thus seem to be the same as or closely associated with indophenol oxidase (3). Indirectly, these results would support Stotz et al. (12) in their belief that no special "ascorbic acid oxidase" is necessary to account for the oxidation of ascorbic acid.

SUMMARY

The use of ascorbic acid as a substrate in oxidase measurements is suggested. The effects of various factors, including the pH value of the substrate, concentration of the substrate, and concentration of the enzyme, were determined. Ascorbic acid was shown to be less injurious to the enzyme than a glucose-derivative substrate. In the case of numerous plant products, the oxidase activity with an ascorbic acid substrate averaged 98.7 percent greater than with a glucose-derivative substrate. This greater sensitivity should permit measurements of smaller changes in oxidase activity of fruits in storage. It should find application in investigations not only of soft scald but also of certain other physiological diseases wherein oxidative changes may be a causative factor. Data are included to show that the oxidation of the ascorbic acid is enzymic and not metallic.

Ascorbic acid substrate possesses the following advantages over a glucose-derivative substrate: (1) Ascorbic acid is a definite chemical compound available in pure form; (2) it is more readily oxidized, enabling the investigator to note smaller differences in enzyme activity; (3) it is water-clear in solution, facilitating titration; (4) it is less injurious to the enzyme; and (5) its use as a substrate saves time, since ascorbic acid is readily soluble in water and a fresh solution may be prepared in a few minutes.

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EFFECTS OF VARIOUS AMOUNTS OF NITROGEN, POTASSIUM, AND PHOSPHORUS ON GROWTH AND ASSIMILATION IN YOUNG APPLE TREES¹

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INTRODUCTION

During the past 25 years it has become generally recognized in the United States that most commercial apple orchards require rather liberal applications of nitrogenous fertilizers to maintain them in optimum production. Experimental results relative to the need for potassium and phosphorus have been less conclusive. Some investigators have reported definite benefits from the use of potassium (10, 14),² while many others have observed no beneficial results from its use (2, 9). Since potassium-deficiency symptoms rarely have been observed on apples in this country, the assumption is generally made that soils contain a sufficient amount of this element to meet the needs of apple orchards.

It is generally agreed that fruit soils of the United States have a sufficient supply of available phosphorus to meet tree requirements. Although there are a few instances on record that suggest a benefit from phosphorus (8), there is no evidence that apple orchards usually respond to applications of phosphorus.

Several investigators have estimated the relative intake of nitrogen, phosphorus, and potassium by apple trees. Van Slyke, Taylor, and Andrews (13) estimated the proportion for bearing trees as 4 parts nitrogen (N_2), 1 part phosphorus pentoxide (P_2O_5), and 4 parts potassium oxide (K_2O). This would approximate 9 parts nitrogen, 1 part phosphorus, and 7 parts potassium. Heinicke (6) reports a relationship for heavily bearing trees of 3.9 nitrogen, 0.5 phosphorus, and 3.5 potassium, or a ratio of approximately 8 : 1 : 7. Thomas (12) reported for nonbearing apple trees receiving an N-P-K fertilizer treatment in cultivation a ratio of 5.72 N_2 , 1.0 P_2O_5 , and 4.44 K_2O , or approximately 13 nitrogen, 1 phosphorus, and 8 potassium.

The above-mentioned investigations dealt with the level of nitrogen, potassium, and phosphorus found in the tissues of apple trees, but this level may not necessarily represent the ratio required for optimum growth. To study further the requirement of young apple trees for these three elements and to determine symptoms of moderate shortage, an experiment was set up in the greenhouse with trees grown in sand cultures. The studies herein reported describe the effect of different concentrations of nitrogen, potassium, and phosphorus upon tree growth and photosynthetic activity of the leaves.

¹ Received for publication August 12, 1939.

² Italic numbers in parentheses refer to Literature Cited, p. 115.

EXPERIMENTAL PROCEDURE

On February 5, 1937, seventy-two 1-year whips of York Imperial apples, grafted on French seedling piece roots, were carefully selected for uniformity in the nursery row and were brought to the greenhouse for potting. These trees were cut back to two buds above the graft union, and the roots were severely pruned in order to remove as much storage tissue as possible. Each tree, after being weighed, was planted in a 3-gallon glazed earthenware crock, in pure washed sand ground to medium fineness. A piece of glass tubing, fitted to a hole in the base of each crock, provided drainage. Distilled water was supplied to all trees until February 25. At that time the buds had developed into shoots approximately 2 inches long. Each tree was trained to a single shoot, and differential treatment was begun.

Full details of the nutrient solution used are given in table 1. Six trees received a complete nutrient solution (table 1, treatment A), containing 168 p. p. m. (parts per million) of nitrogen, 117 p. p. m. of potassium, and 93 p. p. m. of phosphorus, besides an adequate concentration of other elements generally recognized as essential for plant growth. This treatment represented the highest concentration of all three elements under consideration. The remaining 66 trees were divided into 3 series of 22 trees each, designated the nitrogen, potassium, and phosphorus series, respectively. Trees in each series were further divided into groups receiving treatments indicated in table 1. Four trees were included in each treatment with the exception of treatments F, M, and S, these having two trees each. In the nitrogen series the nitrogen content of the nutrient solution ranged from 0 to 60 p. p. m. Likewise, in the phosphorus and potassium series, the concentration of these elements ranged from 0 to 40 and from 0 to 60 p. p. m., respectively. In all series, nutrient elements other than the one varied were kept at full concentrations. The reactions of the freshly prepared nutrient solutions, as determined with the quinhydrone electrode, ranged from pH 5.9 to pH 6.9.

Two quarts of nutrient solution, consisting of the previous day's leaching made up to volume, was supplied daily to each tree. Complete change of nutrient solution and flushing of the sand were effected each week.

Temperature and humidity conditions in the greenhouse where these studies were conducted were subject to rather wide fluctuations. An attempt was made during the first 2 months of the experiment to hold the night temperature to 65° F., with the day temperature between 75° and 80°. During the summer months, alternate strips painted white on the greenhouse roof served to reduce the daytime temperature. During this period temperatures generally ranged between 80° and 105°.

Application of nutrients was discontinued on September 29, 1937, and the trees were harvested November 20. Measurements were made on tree diameter, length of terminal growth, and total linear growth including laterals when present. Total green weight of top was also recorded. Samples of bark, leaves, wood, and roots were analyzed for total organic nitrogen (1, p. 20 [22]) and potassium. Samples for the last-named test were prepared for precipitation according to the method of Sideris (11). The method of precipitation was that of Wilcox (15).

TABLE 1.—Composition of nutrient solutions¹

Series and treatment designation	Amount supplied in nutrient solution			Amount of stock solution per liter of nutrient solution ²						
	N	P	K	1M NaNO ₃	1M KH ₂ PO ₄	1M MgSO ₄	1M CaCl ₂	1M Ca(NO ₃) ₂	1M NaH ₂ PO ₄	1M KCl
Control (full nutrient solution):	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>ML.</i>	<i>ML.</i>	<i>ML.</i>	<i>ML.</i>	<i>ML.</i>	<i>ML.</i>	<i>ML.</i>
A	168	93	117	12.000	3.0	2.0	4.5			
Nitrogen series:										
B	60	93	117	4.260	3.0	2.0	4.5			
C	30	93	117	2.130	3.0	2.0	4.5			
D	15	93	117	1.080	3.0	2.0	4.5			
E	5	93	117	.360	3.0	2.0	4.5			
F	2	93	117	.144	3.0	2.0	4.5			
G	0	93	117	.000	3.0	2.0	4.5			
Potassium series:										
N	168	93	60			2.0		6.0	3.0	1.540
O	168	93	30			2.0		6.0	3.0	.770
P	168	93	10			2.0		6.0	3.0	.260
Q	168	93	4			2.0		6.0	3.0	.104
R	168	93	2			2.0		6.0	3.0	.052
S	168	93	0			2.0		6.0	3.0	.000
Phosphorus series:										
H	168	40	117			2.0		6.0	1.288	3.00
I	168	20	117			2.0		6.0	.644	3.00
J	168	10	117			2.0		6.0	.322	3.00
K	168	4	117			2.0		6.0	.129	3.00
L	168	2	117			2.0		6.0	.064	3.00
M	168	0	117			2.0		6.0	.000	3.00

¹ 0.5 p. p. m. of manganese and boron were supplied in all nutrient solutions. Iron was applied directly to the sand as ferric citrate.

² Nutrient solutions were made up in quantities of 18 to 20 liters; hence the quantities of stock solution per liter are reported to the third decimal.

In order to obtain a measure of the relative rate of growth and ultimate leaf size, daily leaf-area measurements were made on selected leaves from shortly after the time of unfolding until there was no further increase in area. For this purpose, two newly formed leaves on each tree were tagged during May and early June and daily measurements were made by tracing the outlines of the leaves on paper. A planimeter was used to determine the area.

Photosynthetic measurements were made according to the method of Heinicke and Hoffman (7) on individual leaves of typical plants.

EXPERIMENTAL RESULTS

NITROGEN SERIES

Typical trees of the nitrogen series photographed on August 13, are shown in figure 1. These pictures show clearly that, as the nitrogen level was increased in the nutrient solution, growth was correspondingly increased. Trees grown without nitrogen were characterized by rather short, slender stems bearing small yellowish-green leaves with reddish-colored petioles that formed relatively narrow angles with the stem. As the nitrogen content of the nutrient solution was increased, these characteristics became progressively less pronounced. Terminal bud formation on main shoots had not occurred on trees receiving treatments A, B, and C at the time of harvest (November 20), while terminal elongation of trees receiving treatment G had ceased as early as June 2, followed by similar cessation of growth in trees receiving treatments F, E, and D in the order mentioned.

Average growth measurements of trees in the nitrogen series are recorded in table 2. As determined by weight of tops, height of tree, growth of laterals, total linear growth, and diameter of stem, growth was significantly reduced with decreasing supplies of nitrogen in the

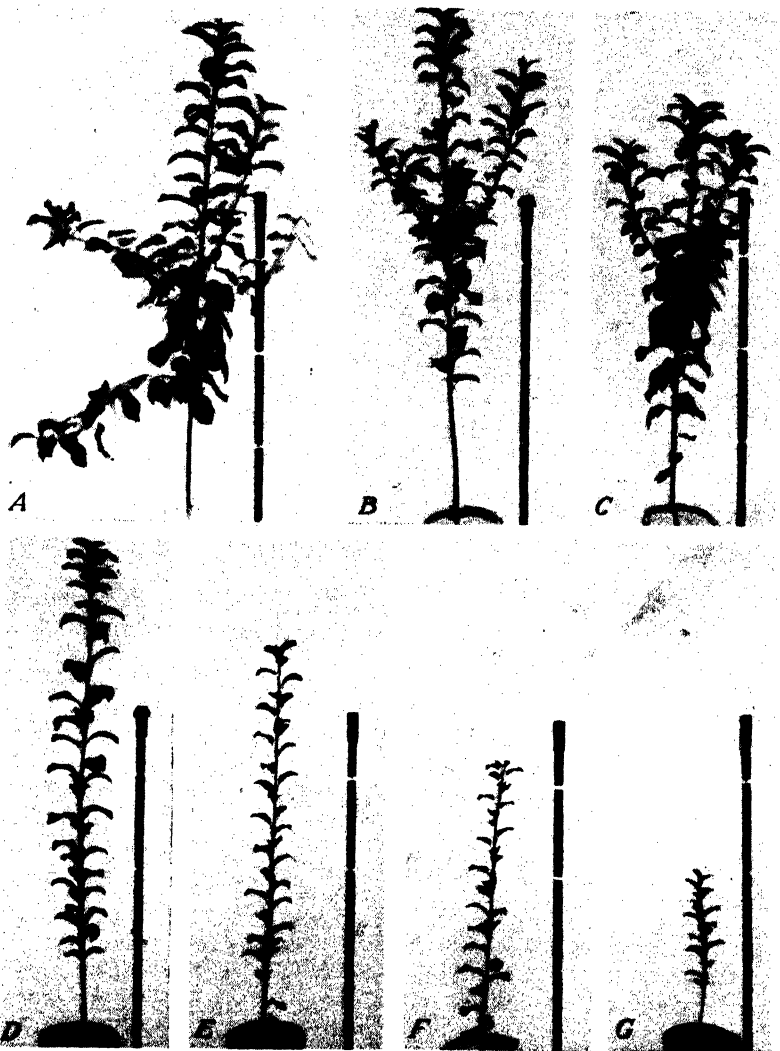


FIGURE 1.—Typical apple trees grown with various amounts of nitrogen in the nutrient solution: A, 168 p. p. m.; B, 60 p. p. m.; C, 30 p. p. m.; D, 15 p. p. m.; E, 5 p. p. m.; F, 2 p. p. m.; G, 0 p. p. m.

nutrient solution. The data clearly indicate that under the conditions of this experiment nitrogen was a limiting factor in growth when supplied in amounts less than 60 p. p. m. Since considerably less difference in growth, as measured by weight of tops, resulted from reducing nitrogen from 168 to 60 p. p. m. than from 60 to 30 p. p. m.,

it is probable that, with the apple, concentrations above 60 p. p. m. approach the luxury range for this element. Reduction below 60 p. p. m. of nitrogen in the nutrient solution reduced the amount of growth almost quantitatively. The greatest growth-rate increase, as measured by weight of tops, was obtained when the nitrogen level of the nutrient solution was advanced from 15 to 30 p. p. m.

TABLE 2.—*Effect of different concentrations of nitrogen, potassium, and phosphorus on growth of apple trees*

Series and treatment designation	Amount of N, P, or K in nutrient solution	Mean green weight of tops ¹	Mean height	Mean total length of laterals	Mean total linear growth	Mean diameter of trunk	CO ₂ assimilated per 100 cm. ² ²
Nitrogen series:	P. p. m.	Gm.	Cm.	Cm.	Cm.	Cm.	Mg.-hrs
A ³	168	341	181.3	408.4	589.7	1.67	23.5
B.....	60	313	175.3	348.1	523.4	1.66	22.7
C.....	30	232	178.9	172.3	351.2	1.42	15.8
D.....	15	106	157.4	.0	157.4	1.05	15.0
E.....	5	51	117.8	.0	117.8	.74	12.0
F.....	2	34	69.8	.0	69.8	.64	7.4
G.....	0	31	62.4	.0	62.4	.51	6.7
Potassium series:							
A ³	117	341	181.3	408.4	589.7	1.67	23.6
N.....	60	314	184.1	297.7	481.8	1.66	19.8
O.....	30	227	170.2	144.8	315.0	1.39	19.0
P.....	10	197	175.6	78.4	254.0	1.33	17.8
Q.....	4	104	148.7	.0	148.7	1.06	17.0
R.....	2	84	137.6	.0	137.6	.98	14.1
S.....	0	51	111.4	.0	111.4	.78	13.6
Phosphorus series:							
A ³	93	341	181.3	408.4	589.7	1.67	20.4
IL.....	40	369	186.1	415.2	601.3	1.73	19.7
L.....	20	355	168.3	382.1	550.4	1.67	19.3
J.....	10	365	193.2	467.3	660.5	1.68	20.3
K.....	4	349	197.8	342.9	540.7	1.65	20.1
L.....	2	216	188.6	103.8	292.4	1.35	19.2
M.....	0	37	107.6	.0	107.6	.62	.2

¹ Differences between means required for significance according to Fisher's (4) method of analysis of variance are as follows: Nitrogen series, 52.9; potassium series, 60.5; phosphorus series, 54.8.

² Average of 24 or more determinations on 12 typical leaves of each treatment.

³ Control (full nutrient solution).

The cumulative area of typical leaves of the nitrogen series is graphically illustrated in figure 2, which shows that the rate of growth, as well as the ultimate size, was markedly affected by the amount of nitrogen supplied in the nutrient solution. It is interesting to note that with all treatments approximately the same amount of time (12 to 15 days) was required for the leaves to reach maturity. It should be pointed out that the differences in leaf area represented in figure 2 are relative values for leaf growth at the time these measurements were made and are not representative of the entire leaf system of the trees. Difference between treatments in leaf size was considerably less earlier in the season because of the nitrogen reserve in the plant tissue prior to differential treatment. The no-nitrogen treatment is not included in figure 2 because terminal bud formation was under way when these measurements were started.

Not only was the total leaf area greater with increasing nitrogen supplied in the nutrient solution, but the apparent rate of photosynthesis per unit area was also greater (table 2). Carbon dioxide assimilation was measured simultaneously on representative leaves of trees receiving each treatment. The values in table 2 are averages of 24 or more determinations on 12 typical leaves for each nitrogen concentration. These data show that the rate of apparent photo-

synthesis was roughly proportional to the amount of nitrogen supplied, and that the average assimilation of leaves receiving 168 p. p. m. of nitrogen was 3.5 times that of leaves receiving no nitrogen. Significant differences were also obtained between treatments relatively high in nitrogen. Thus it may be seen that leaves receiving 60 p. p. m. assimilated 44 percent more carbon dioxide than leaves receiving 30 p. p. m.

Childers and Cowart (3) report similar results between assimilation rates of trees receiving full nutrient solution as compared with no-

nitrogen treatments. Heinicke and Hoffman (7), comparing light- and dark-green leaves under field conditions, obtained three times the carbon dioxide assimilation in the case of the latter.

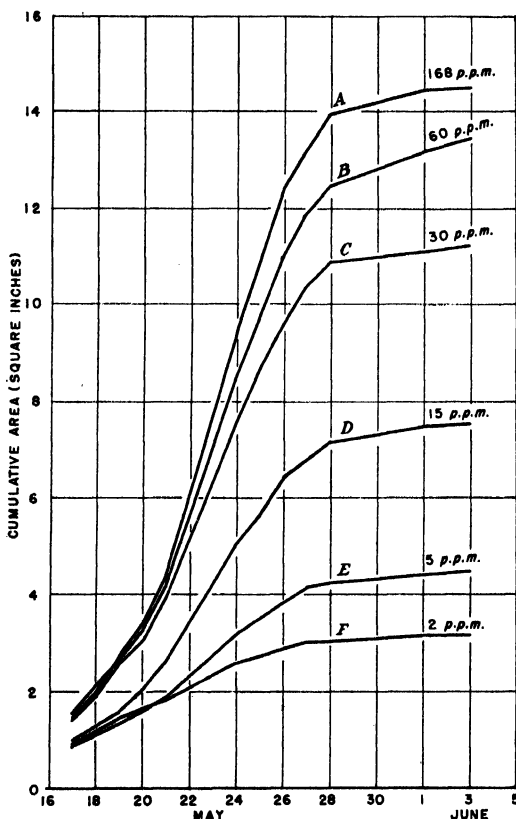


FIGURE 2.—Cumulative area of typical leaves of apple trees grown with various amounts of nitrogen in the nutrient solution.

season the lower leaves of trees receiving the 2- and 4- p. p. m. treatments developed typical marginal scorching.

Growth measurements of trees in the potassium series are included in table 2. The trend of all measurements was strikingly similar to that in the nitrogen series. Growth, measured by weight of top, length of shoots, and diameter of stem, was roughly proportional to the amount of potassium supplied in the nutrient solution. The greatest increase occurred between 4 and 10 p. p. m. Even though visible symptoms of potassium deficiency did not occur on trees

POTASSIUM SERIES

Typical trees of the potassium series are shown in figure 3. It is to be seen here that a direct relationship existed between the amount of potassium supplied in the nutrient solution and growth response.

Trees receiving no potassium exhibited definite deficiency symptoms by early May. The lower leaves of these trees became light green in color, and later developed a rather pronounced chlorosis. This condition, in turn, was followed by typical scorching around the leaf margins. Later in the

receiving 10 p. p. m. or more, growth increased with increasing concentrations.

The effect of the potassium treatment on the growth of young leaves in early June is graphically illustrated in figure 4. The effect



FIGURE 3.—Typical apple trees grown with various amounts of potassium in the nutrient solution: A, 117 p. p. m.; N, 60 p. p. m.; O, 30 p. p. m.; P, 10 p. p. m.; Q, 4 p. p. m.; R, 2 p. p. m.; S, 0 p. p. m.

is less pronounced than in the nitrogen series, but the trend is the same. However, it will be noted that, at a concentration of 30 p. p. m. or less, the rate of growth and ultimate size of leaf are considerably greater than in leaves receiving the same concentrations of nitrogen.

PHOSPHORUS SERIES

Decreasing amounts of phosphorus supplied in the nutrient solution had no effect on tree growth (fig. 5) until a concentration of less than 4 p. p. m. was reached. At 2 p. p. m. of phosphorus, the trees did not exhibit any of the usual deficiency symptoms; however, the growth measurements show a significant downward trend as compared with those of trees receiving higher concentrations of the element (table 2 and fig. 6). The no-phosphorus trees were typically

short and slender, with small dark-green leaves of a purplish cast. Symptoms closely resembled those of the no-nitrogen trees except for the color of foliage.

These results indicate that the phosphorus requirement of apple trees is very low as compared with the requirements for nitrogen and potassium. Because of the numerous failures to obtain response to phosphorus under field conditions, it would seem that soils generally have available a sufficient supply of this element to meet the relatively small needs of the apple tree.

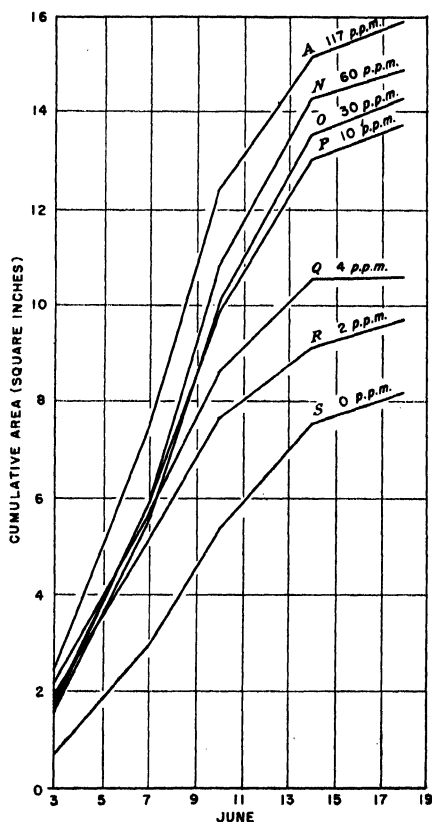


FIGURE 4.—Cumulative area of typical leaves of apple trees grown with various amounts of potassium in the nutrient solution.

Likewise, in the potassium series the potassium content of the tissues generally increases with increasing concentrations of this element in the nutrient solution and in turn is directly associated with growth response.

The data in table 3 show clearly that the potassium content of leaves and bark in the nitrogen series and the nitrogen content of these tissues in the potassium series are independent of differential treatment. Thus it may be seen that the potassium content is virtually constant throughout the nitrogen series. Similarly, the nitrogen

NITROGEN AND POTASSIUM ANALYSES

The nitrogen and potassium analyses of plant tissue in the nitrogen and potassium series are summarized in table 3. In the nitrogen series the nitrogen content of both leaf and bark tissue is directly related to the concentration of this element in the nutrient solution. With wood and root tissue, some discrepancies are apparent but the trend is roughly the same.

content of the leaves and bark in the potassium series is relatively constant, with the potassium ranging from 0 to 117 p. p. m. It would therefore seem that within the limits of these concentrations there does not exist an unfavorable balance between nitrogen and potassium.

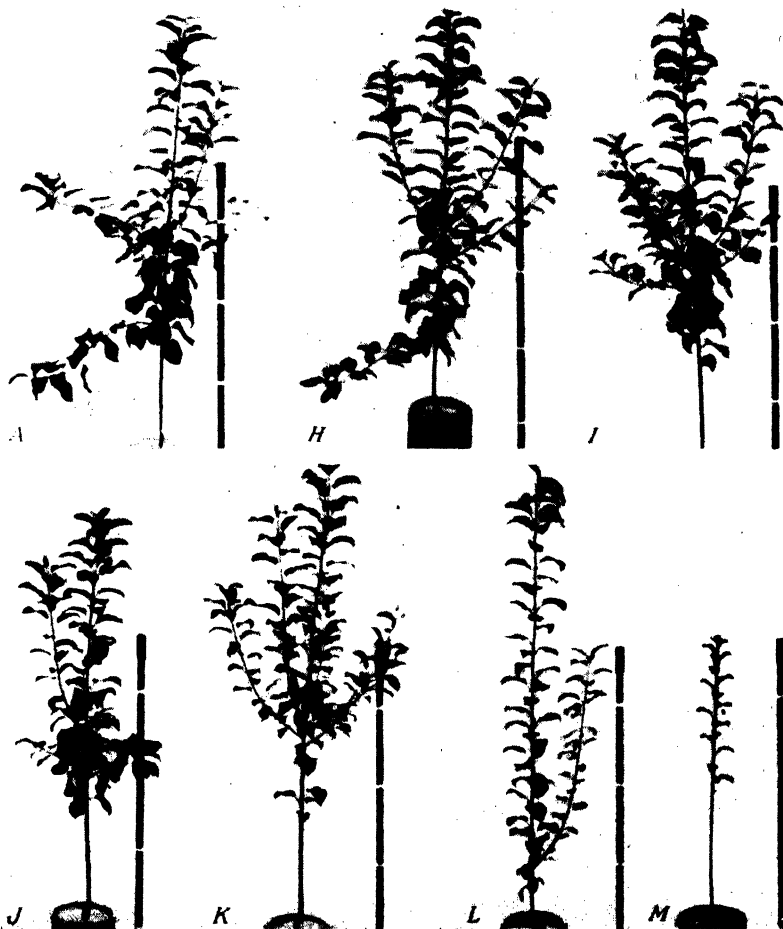


FIGURE 5.—Typical apple trees grown with various amounts of phosphorus in the nutrient solution: A, 93 p. p. m.; H, 40 p. p. m.; I, 20 p. p. m.; J, 10 p. p. m.; K, 4 p. p. m.; L, 2 p. p. m.; M, 0 p. p. m.

LEAF ASSIMILATION AS AFFECTED BY INCREASING THE NITROGEN CONTENT OF THE NUTRIENT SOLUTION

On August 23, after terminal growth had ceased at the lower nitrogen levels, two trees from each of the 2-, 5-, and 15-p. p. m. treatments of the nitrogen series (fig. 7) were selected for determining the effect of increasing the nitrogen content of the nutrient solution on carbon dioxide assimilation. Preliminary measurements (table 4) were made simultaneously on selected leaves of each tree to determine the relative rate of carbon dioxide assimilation. On August 28, one tree of each

pair (trees 4, 2, and 6) received a complete nutrient solution containing 168 p. p. m. of nitrogen. The other tree of each pair (trees 3, 1, and 5) continued to receive the usual treatment. From August 31 to September 29, three series of determinations were made which are summarized in table 4. A typical tree, receiving a complete nutrient solution (168 p. p. m. of nitrogen) throughout the season, was included in this experiment to serve as a basis for comparison.

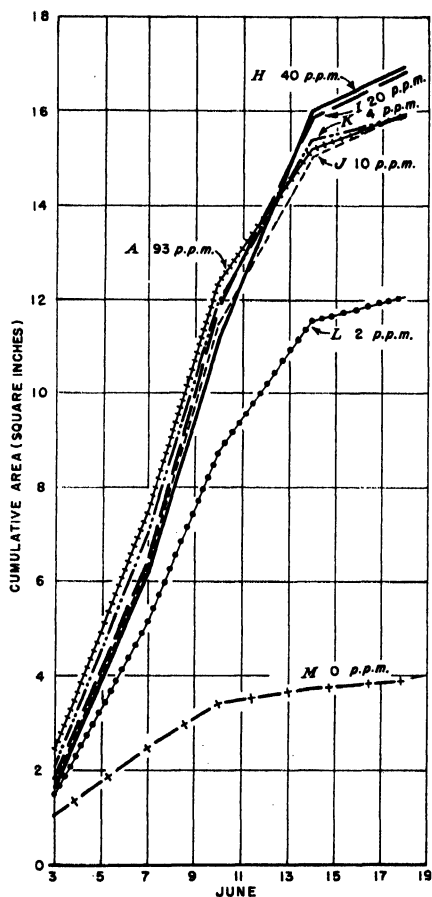


FIGURE 6.—Cumulative area of typical leaves of apple trees grown with various amounts of phosphorus in the nutrient solution.

after August 28 was still active several weeks after their companion trees were completely defoliated (fig. 8).

The increase of nitrogen intake as a result of increasing the supply of nitrogen in the nutrient solution is clearly indicated in table 4. On September 28, the nitrogen content of the foliage of trees 2, 4, and 6 had equalled or exceeded that of tree 7, which had received a full nutrient solution throughout the season. It is interesting to note (table 4) that the rate of increase (as compared with their com-

The foliage of all trees receiving full nutrient solution on August 28 became noticeably greener within a few days after the application was made. From August 31 to September 8 there was a definite increase in rate of photosynthesis of these trees as compared with their companion trees, which continued to receive the usual concentration. During this period, tree 6, which formerly received 2 p. p. m. of nitrogen, more than doubled its rate of assimilation as compared with its control tree (table 4). The data in table 4 clearly show that in all cases the rate of assimilation of the foliage was increased by increasing the nitrogen content of the nutrient solution. Approximately 1 month after additional nitrogen was added, the photosynthetic rate of tree 6 had increased more than threefold as compared with tree 5. Tree 2 had almost doubled its rate, and tree 4 had increased by two-thirds as compared with its companion tree. Heinicke (5) reports similar increases in assimilation as a result of late summer applications of nitrogen under field conditions. Besides a large increase in rate of assimilation the foliage of trees receiving the full nutrient solution

panion trees) in the nitrogen content of the leaves of these trees was closely proportional to the percentage increase in assimilation. Tree 2 had a nitrogen content of 1.9 times that of its companion tree (table 4) and it also assimilated 1.9 times as much carbon dioxide (table 4, September 20 to 29) as its companion tree. Trees 4 and 6 show similar relationships between nitrogen content of foliage and rate of assimilation as compared to their respective companion trees.

TABLE 3.—*Summary of nitrogen and potassium analyses*

Series and treatment designation	N contained in nutrient solution	K contained in nutrient solution	N content of—				K content of—	
			Leaves, Oct. 25	Bark, Nov. 20	Roots, Nov. 20	Wood, Nov. 20	Leaves, Oct. 25 ¹	Bark, Nov. 20
Nitrogen series:	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
A ²	168	117	1.856	1.488	1.256	0.555	2.31	1.049
B.....	60	117	1.702	1.179	.636	.367	2.35	1.053
C.....	30	117	1.392	1.008	1.072	.346	2.29	1.122
D.....	15	117	1.229	.939	1.107	.369	2.35	1.144
E.....	5	117	1.148	.801	.763	.315	1.93	.861
F.....	2	117	.940	.776	.948	.310	2.00	1.018
G.....	0	117573	.357	.182
Potassium series:								
A ²	168	117	1.856	1.488	2.31	1.049
N.....	168	60	1.839	1.416	1.95	.953
O.....	168	30	1.819	1.581	1.69	.815
P.....	168	10	1.814	1.40183	.682
Q.....	168	4	1.786	1.69168	.740
R.....	168	2	1.649	1.78766	.712
S.....	168	0	1.620	2.08433	.639

¹ Differences between leaf values of different treatments required for significance, according to Fisher's (4) method of analysis of variance, are as follows: Nitrogen series, 0.121 for nitrogen and 0.322 for potassium; potassium series, 0.218 for nitrogen and 0.228 for potassium.

² Control (full nutrient solution).

TABLE 4.—*Effect of nitrogen increase in nutrient solution on nitrogen content and CO₂ assimilation of apple leaves*

Tree No.	Nitrogen in nutrient solution prior to Aug. 28	Nitrogen in nutrient solution after Aug. 28	CO ₂ assimilated per 100 cm. ² Aug. 23-28 ¹	CO ₂ assimilated ²			Nitrogen content ³ of—			
				Aug. 31-Sept. 8	Sept. 9-18	Sept. 20-29	Leaves, Sept. 28	Bark, Nov. 20	Wood, Nov. 20	Roots, Nov. 20
	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>Mg. hrs.</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
7.....	168	168	16.0	121.7	122.0	145.0	2.10	1.50	0.57	0.91
3.....	15	15	11.4	126.3	122.4	99.7	1.37	.77	.28	1.19
4.....	15	168	9.5	145.7	157.2	165.3	2.07	2.19	.85	1.54
1.....	5	5	9.3	116.3	90.0	105.0	1.15	.81	.34	.77
2.....	5	168	7.0	146.0	172.0	199.7	2.18	1.65	.96	1.72
5.....	2	2	4.2	81.0	72.2	68.7	.94	.70	.23	.91
6.....	2	168	4.3	169.3	272.8	230.0	2.58	1.88	.89	1.54

¹ Each value is an average of 6 determinations made on 2 leaves of each tree. The same 2 leaves on each tree were used throughout the test.

² Expressed as percentage of CO₂ assimilated from Aug. 23 to 28.

³ Percentage of dry weight.

Data on nitrogen content of bark, wood, and roots of trees included in this experiment are shown in table 4 and clearly indicate large increases in nitrogen content of these tissues as a result of nitrogen increase in the nutrient solution. Tree 7 was still making active terminal growth at the time these samples were taken, which perhaps accounts for the relatively low nitrogen content of the wood and roots of this tree.

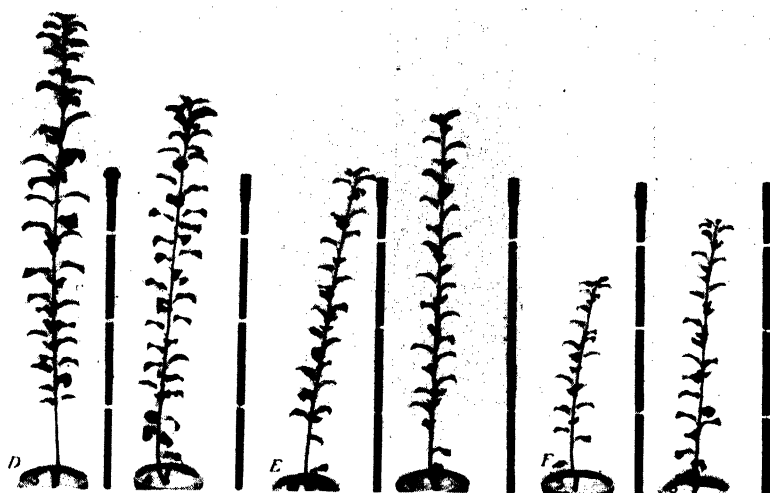


FIGURE 7.—Apple trees grown with various amounts of nitrogen in the nutrient solution; treatment D, 15 p. p. m. (trees 3 and 4); treatment E, 5 p. p. m. (trees 1 and 2); treatment F, 2 p. p. m. (trees 5 and 6). Photographed August 23.



FIGURE 8.—Same trees as shown in figure 7. Trees 4, 2, and 6 received complete nutrient solution beginning August 28. Photographed September 29.

DISCUSSION

It should be emphasized that the concentration of nutrients used in these tests represents the amounts in the water added to the sand. Since many orchard soils hold from 20 to 30 percent of water, it seems probable that 15 p. p. m. based on a soil analysis would roughly approximate 60 p. p. m. in the water as used here.

Under the conditions of this test, significant differences in growth in the nitrogen series were obtained when the nitrogen was reduced from 60 to 30 p. p. m. A slight increase in growth occurred between 60 and 168 p. p. m., but this was less than was required for significance.

In the potassium series, also, significant differences in green weight of top occurred when the potassium content was reduced from 60 to 30 p. p. m. This would indicate that approximately similar concentrations of available potassium and available nitrogen are required for optimum growth of young apple trees.

It is interesting to note the effect of nutrient concentration upon lateral branching. In the nitrogen series the development of laterals did not occur below the 30 p. p. m. treatment; in the potassium series there was no branching below 10 p. p. m.

The wide variation in the potassium content of the leaves of trees receiving different amounts of potash is particularly interesting. With 30 p. p. m. of potassium in the nutrient solution, leaves showed 1.69 percent of potassium on a dry-weight basis. With this concentration in the leaves, growth was significantly reduced when all other nutrients were available in ample quantities. The analyses of leaves from the 4-p. p. m. treatment showed a reduction to 0.68 percent of potassium on a dry-weight basis. This wide fluctuation in the content of the leaves from trees furnished with various amounts of this element suggests the value of determining the potassium content of the leaves as a measure of potassium availability under field conditions. In these tests, trees responded to additional potassium when leaf analyses showed 1.69 percent of potassium present. It is probable, of course, that had the nitrogen also been reduced on these trees, no response from additional potash would have been observed. Under orchard conditions nitrogen is usually a limiting factor in obtaining maximum growth, and response to potash applications could not be expected until the full nitrogen requirement had been supplied, unless the potash availability was extremely low.

The fact that definite symptoms of potassium deficiency were not observed until the potash content of the nutrient solution was reduced to less than 10 p. p. m. indicates that response to potash applications may be expected under some conditions where no visible symptoms of deficiency can be observed.

In the phosphorus series, significant reduction in growth of trees did not occur until the phosphorus content of the nutrient solution was reduced to less than 4 p. p. m. In the present work, the phosphorus concentration required for optimum growth was approximately only one-fifteenth of the concentration required for nitrogen and potassium. Thus this study substantiates results of earlier workers in indicating that the phosphorus requirement of apple trees is very low as compared with nitrogen and potassium.

The fact that the potassium concentration in the nitrogen series did not increase as growth of trees was reduced by lack of nitrogen

indicates that marked luxury storage of potassium under these conditions did not occur. Similarly, the fact that the nitrogen content of the trees in the potassium series where growth was reduced by lack of potassium did not increase significantly indicates that luxury absorption of nitrogen did not occur under these conditions. Neither the absorption of nitrogen in the potassium series nor the absorption of potassium in the nitrogen series was appreciably affected by varying the concentration of the other element.

The results of photosynthetic measurements on the trees in the nitrogen series substantiate results of earlier work in showing a marked decrease in photosynthetic activity of the leaves under reduced nitrogen conditions. The photosynthetic measurements on the trees in the potassium series indicate that the effects of decreased potassium on leaf efficiency are similar to but less marked than the effects of decreased nitrogen.

SUMMARY

One-year York Imperial apple trees were grown in the greenhouse in sand cultures in a study of the effects of various concentrations of nitrogen, potassium, and phosphorus upon growth response and photosynthetic activity of leaves. The following concentrations were used: Nitrogen, 0, 2, 5, 15, 30, 60, and 168 p. p. m.; potassium, 0, 2, 4, 10, 30, 60, and 117 p. p. m.; and phosphorus, 0, 2, 4, 10, 20, 40, and 93 p. p. m. In all series, nutrient elements other than the one studied were kept at full concentration.

Trees grown with nitrogen at 60 p. p. m. made somewhat less growth, as measured by total linear growth and total fresh weight, than trees grown with 168 p. p. m., though the differences were not statistically significant. Concentrations of nitrogen below 60 p. p. m. reduced the amount of growth almost quantitatively, the largest difference in growth resulting at concentrations between 15 and 30 p. p. m. Potassium at 60 p. p. m. gave less growth than at 117 p. p. m., and less growth with each decreasing concentration. The greatest difference in growth occurred between 4 and 10 p. p. m. Growth with 2, 4, 10, and 30 p. p. m. was not decreased as much as with corresponding concentrations of nitrogen. Definite deficiency symptoms in the potassium series did not occur on trees receiving 10 p. p. m. or more, although growth increased with increasing concentrations until more than 60 p. p. m. were supplied. In the phosphorus series, growth was approximately uniform in all treatments receiving 4 p. p. m. or more of this element. Only when phosphorus was completely lacking did visible symptoms of deficiency occur.

The nitrogen and potassium content of the plant tissues, particularly of the leaves and bark, increased with increasing concentrations of these elements in the nutrient solution. The potassium content of leaves and bark in the nitrogen series and the nitrogen content of these tissues in the potassium series were found to be independent of differential treatment.

The rate of photosynthesis per unit of leaf area was markedly reduced in the nitrogen series with decreasing amounts of this element in the nutrient solution. A similar but less marked decrease occurred in the potassium series. A significant decrease in assimilation occurred in the phosphorus series only with the complete absence of this element.

Trees grown at a low nitrogen level until the terminal bud had formed were supplied with a high nitrogen solution in late August. Carbon dioxide assimilated per unit area of leaf was measured periodically prior to and immediately following the application of the higher nitrogen solution. Leaves became noticeably greener within a few days after additional nitrogen was supplied. Carbon dioxide assimilation per unit area of leaf significantly increased during this period, and 21 days after application of increased nitrogen it had doubled in most cases. The increase in nitrogen content of the leaves was closely correlated with the increase in assimilation.

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INHERITANCE IN THE CUCUMBER¹

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INTRODUCTION

In connection with breeding work conducted during the past few years, studies have been made on the inheritance of several characters in the cucumber *Cucumis sativus* L. The results of a portion of these studies are reported in this paper.

MATERIALS AND METHODS

The data herein presented were obtained from a study of the F_1 and F_2 progenies of a cross made in 1934 between two inbred cucumber parents that differed in certain characters as shown in table 1.

TABLE 1.—*Contrasting characters of two inbred cucumber parents*

Characters	Parents	
	Male	Female
Growth habit.....	Determinate.	Indeterminate.
Height of plant.....	Short.	Tall.
Spine color.....	Black.	White.
Spine size.....	Fine.	Coarse.
Spine frequency.....	Numerous.	Few.
Mature fruit color.....	Red.	Cream.
Mature fruit netting.....	Heavy.	None or very slight
Number of mature fruits per plant.....	Few.	Many.
Total weight of mature fruit per plant.....	Small.	Large.
Average weight (size) of mature fruit per plant.....	do.	do.
Total number of fruit per plant (mature + green).....	Few.	Many.
Average weight of each fruit per plant (mature + green).....	Small.	Large.
Days after planting to first male flower.....	Few.	Many.
Days after planting to first female flower.....	Many.	Few.
Number of female flowers per plant.....	Few.	Many.
Number of laterals per plant.....	do.	do.
Average length of lateral per plant.....	Short.	Long.
Total length of all laterals per plant.....	Small.	Large.

Of the characters mentioned in table 1, determinate growth habit requires, perhaps, further explanation. In a strain of cucumbers received from the Union of Soviet Socialist Republics in 1928, a plant appeared which was determinate in growth habit. In this plant and succeeding inbred progenies derived from it, the apical growth terminated in a cluster of male or female flowers, the laterals were few and very short, and plant height ranged from 6 to 12 inches depending upon environmental conditions (fig. 1).

In the F_1 and F_2 populations, grown in the greenhouse, all female flowers produced were hand-pollinated and the fruits were permitted to remain on the plant until the plant appeared to have reached its

¹ Received for publication Mar. 4, 1939. Paper No. 1693 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station. Completion of certain parts of this paper was made possible by personnel of Works Projects Administration official project No. 665-71-3-69.

production capacity and started to die, when all fruits and the plant itself were harvested and final measurements taken. F_3 lines representing each F_2 plant were grown in the field and the plants treated in the same manner as the F_2 plants except that open pollination was allowed to take place.



FIGURE 1.—Dwarf determinate cucumber parent in foreground; indeterminate parent in background

GENETIC ANALYSIS

The ratios obtained for the various characters in the F_2 population, which consisted of 151 plants in the plant height study and 156 plants in all others, are given in table 2.

TABLE 2.—Differential characters of the two parents, and appearance of the F_1 and of the segregates obtained in the F_2 of a cucumber cross

Characters	Parents		F_1	F_2 segregates ¹	χ^2 test for goodness of fit; P lies between— ²
	Male	Female			
Growth habit.....	Determinate	Indeterminate	Determinate	36 determinate ³ 81 segregating ³ 39 indeterminate ³ (31 tall ³ 79 intermediate ³ 41 short ³ 45 black ³ 69 segregating ³ 42 white ³ 35 coarse ³ 82 segregating ³ 39 fine ³ 35 few ³ 82 segregating ³ 39 numerous ³ (85 red ⁴ 29 orange ⁴ 32 yellow ⁴ 10 cream ⁴ 40 heavy ³ 77 segregating ³ 39 none or very slight ³	0.80 and 0.90 .30 and .50 .30 and .50 .70 and .80 .70 and .80 .90 and .95 .98 and .99
Plant height.....	Short	Tall	Intermediate		
Spine color.....	Black	White	Black or brown		
Spine size.....	Fine	Coarse	Intermediate		
Spine frequency.....	Numerous	Few	Intermediate		
Mature fruit color...	Red	Cream	Red		
Mature fruit netting.	Heavy	None or very slight.	Heavy		

¹ Classified according to the behavior of F_3 lines.

² Involving a 1 : 2 : 1 ratio.

³ Italic numbers in parentheses refer to Literature Cited, p. 127.

⁴ Fisher's table of χ^2 (5).⁵

⁵ Involving a 9 : 3 : 3 : 1 ratio.

GROWTH HABIT

From table 2 it can be seen that the F_1 progeny from the cross between determinate (II) and indeterminate (*ii*) types was determinate. In the F_2 , 117 determinate and 39 indeterminate (*ii*) plants were obtained. Of the 117 determinate plants, 36 proved to be homozygous determinate (II) and 81 heterozygous (*Ii*) in the F_3 generation. Similar results would be expected if the difference between the parents was due to a single factor.

A similar type of determinate growth in the tomato has been reported by Yeager (8) and Currence (2) as differing by a single factor from the indeterminate habit. Currence found that the determinate habit behaved as a recessive in the tomato, which is in contrast to the behavior of this character in the cucumber.

PLANT HEIGHT

The difference between the two parents in plant height appears to be dependent on a single factor in this cross. As shown in table 2, the F_1 was intermediate. In the F_2 , 31 tall (*TT*), 79 intermediate (*Tt*), and 41 short (*tt*) were obtained.

SPINE COLOR

As shown in table 2, the spines on the fruits of F_1 plants were classified as black or brown. In the F_2 , black or brown spines were observed on 114 plants and white spines (*bb*) on 42. In the F_3 , 45 of the 114 plants having black or brown spines proved to be homozygous (*BB*) for black spines and 69 heterozygous (*Bb*) for spine color. The results suggest that a single factor difference existed between the parents for spine color. These results agree with those reported by Wellington (7), Strong (4), and Tkachenko (5).

SPINE SIZE

As indicated in table 2, spine size was intermediate in the F_1 . In the F_2 , coarse or intermediate spines were observed on 117 plants and fine spines (*ff*) on 39 plants. In the F_3 , 82 of the 117 plants having coarse or intermediate spines segregated for spine size and 35 proved to be homozygous (*FF*) for coarse spines. This suggests that a single factor difference existed between the parents for spine size. These results agree with those reported by Wellington (7). Strong (4), from observations made on the F_2 progeny of a cucumber cross, Vickery \times Everyday, suggested that spine size probably was inherited on a multiple factor basis.

SPINE FREQUENCY

In the F_1 of this cross, frequency or number of spines appeared to be intermediate. As shown in table 2, the F_2 consisted of 117 plants having few or an intermediate number of spines and 39 plants having numerous (*ss*) spines. In the F_3 , 82 of the 117 plants having few or an intermediate number of spines segregated and 35 proved homozygous (*SS*) for few spines. It is suggested that a single factor difference existed between the parents for spine frequency, which agrees with the results obtained by Wellington (7), Strong (4), and Tkachenko (5).

MATURE FRUIT COLOR

In studying the inheritance of mature fruit color, the color was found to be red in the F_1 . As shown in table 2, the ratio obtained in the F_2 very closely approaches the theoretical 9:3:3:1 ratio that would be expected on the basis of a two-factor difference between the parents. The four classes obtained were red (RC), orange (Rc), yellow (rc), and cream (rc). Homozygous F_3 lines were obtained for all these colors. The data indicate that the red character is due to both factors being present in a dominant ($RRCC$) condition; the cream to both factors being present in a homozygous recessive condition ($rrcc$); and the orange and yellow to one factor being present in the homozygous recessive and the other factor in either the homozygous dominant or heterozygous condition. In the greenhouse, the yellow is a bright canary yellow, readily distinguished from the cream. In the field, the yellow closely approaches the cream and is often difficult to distinguish from it.

MATURE FRUIT NETTING

From table 2 it can be seen that fruit of the F_1 plants obtained from the cross between the heavy-netted (III) and no-netted (hh) types was heavily netted. In the F_2 the fruits of 117 plants showed heavy or intermediate netting and 39 slight or no netting (hh). In the F_3 , 40 plants of the 117 showing heavy or intermediate netting proved to be homozygous for heavy netting (III) and 77 segregated for this character. It is suggested that a single factor difference existed between the two parents for mature fruit netting, which agrees with the results of Tkachenko (5).

INTERACTION OF GROWTH HABIT AND PLANT HEIGHT

The determinate growth and the plant height genes appear to exert considerable influence on each other as shown to some extent in table 3, where the phenotypic classes obtained in the interaction of the two factors in the F_2 , together with the height range of the phenotypic classes, is given.

TABLE 3.—Height range in each of four phenotypic classes obtained in the F_2 progeny of a cross between tall-indeterminate and short-determinate cucumber lines

Phenotypic classes	Segregation obtained in the F_2	
	Observed	Height class
	Number	Inches
Determinate-tall (IT)	80	19-110
Determinate-short (It)	32	6-19
Indeterminate-tall (iT)	30	71-136
Indeterminate-short (it)	9	32-71

The segregation given in table 3 agrees with the theoretically expected 9:3:3:1 dihybrid ratio. Of interest is the effect of the interaction of the genes I -determinate and T -tall on height of plant. When the gene I for determinate growth habit is present together with the gene t for short plant height, the plants are very short. When I is present with the gene T for tall plant height, the plants are consider-

ably taller. When the indeterminate growth gene *i* is present, both the *T* and *t* plants are considerably taller than in the comparable classes of *I* plants. These data indicate that the *I* factor for determinate growth, when present, tends to decrease the height of the plant. The *I* gene exhibits partial dominance in this respect. The *II* plants are shorter than the *Ii* plants. Attention has been called to the intermediate condition of the *Tt* plants as compared to the parents.

TESTS FOR INDEPENDENT ASSORTMENT

Several characters just discussed were tested in all possible combinations for independent assortment by the use of the χ^2 test for independence. The results of these tests between the independently assorted characters and between characters where the data, although indicating association, do not appear to be sufficient to warrant definite conclusions are presented in table 4. The ratios were determined on 156 F_2 plants checked by F_3 lines from each of these plants.

TABLE 4.—Tests for independent assortment of seven factors responsible for six characters under observation

Parents	F_2 plants in indicated phenotypic classes	χ^2 test for independent assortment; P lies between—
	<i>Number</i>	
Coarse, white spines \times fine, black spines (<i>FFbb</i> \times <i>ffBB</i>).	<i>BF</i> , 85; <i>Bf</i> , 29; <i>bF</i> , 32; <i>bf</i> , 10	0.80 and 0.90
Few, white spines \times numerous, black spines (<i>SSbb</i> \times <i>ssBB</i>).	<i>BS</i> , 85; <i>Bs</i> , 29; <i>bS</i> , 32; <i>bs</i> , 10	.80 and .90
White spines, indeterminate \times black spines determinate (<i>bbit</i> \times <i>BBII</i>).	<i>BI</i> , 86; <i>Bi</i> , 28; <i>bI</i> , 31; <i>bi</i> , 11	.80 and .90
Coarse spines, indeterminate \times fine spines, determinate (<i>FFii</i> \times <i>ffII</i>).	<i>FI</i> , 86; <i>Fi</i> , 31; <i>fI</i> , 31; <i>fi</i> , 8	.30 and .50
Few spines, indeterminate \times numerous spines, determinate (<i>SSii</i> \times <i>ssII</i>).	<i>SI</i> , 86; <i>Si</i> , 31; <i>sI</i> , 31; <i>si</i> , 8	.30 and .50
No netting, coarse spines \times heavy netting, fine spines (<i>hhFF</i> \times <i>HHff</i>).	<i>HfF</i> , 86; <i>hF</i> , 31; <i>Hf</i> , 31; <i>hf</i> , 8	.30 and .50
No netting, few spines \times heavy netting, numerous spines (<i>hhSS</i> \times <i>HHss</i>).	<i>HsS</i> , 86; <i>hS</i> , 31; <i>Hs</i> , 31; <i>hs</i> , 8	.30 and .50
No netting, indeterminate \times heavy netting, determinate (<i>hhii</i> \times <i>HHII</i>).	<i>HI</i> , 89; <i>hI</i> , 28; <i>HI</i> , 28; <i>hi</i> , 11	.50 and .70
Coarse spines, cream fruit \times fine spines, red fruit (<i>FFrrcc</i> \times <i>ffRRCC</i>).	<i>FRc</i> , 58; <i>fRC</i> , 28; <i>FRe</i> , 27; <i>fRe</i> , 1; <i>FrC</i> , 22; <i>frC</i> , 10; <i>Frc</i> , 10; <i>frC</i> , 0.	.01
Few spines, cream fruit \times numerous spines, red fruit (<i>SSrrcc</i> \times <i>ssRRCC</i>).	<i>SRc</i> , 58; <i>sRC</i> , 28; <i>SRe</i> , 27; <i>sRe</i> , 1; <i>SrC</i> , 22; <i>srC</i> , 10; <i>Src</i> , 10; <i>src</i> , 0.	.01
Indeterminate, cream fruit \times determinate, red fruit (<i>ffrrcc</i> \times <i>IIIRCC</i>).	<i>IRc</i> , 64; <i>irc</i> , 22; <i>IRc</i> , 22; <i>irc</i> , 6; <i>IrC</i> , 25; <i>irC</i> , 7; <i>Irc</i> , 6; <i>irc</i> , 4.	.50 and .70

¹ Fisher's table of χ^2 (3).

INDEPENDENTLY ASSORTED CHARACTERS

In table 4, *P* values within the range 0.1 to 0.9 suggest no significant association between the characters for which they were obtained (3). Therefore, the following characters appear to be inherited independently of each other: Spine color and spine size; spine color and spine frequency; spine color and growth habit; spine size and growth habit; spine size and mature fruit netting; spine frequency and growth habit; spine frequency and mature fruit netting; growth habit and mature fruit color; and growth habit and mature fruit netting.

SPINE SIZE AND FREQUENCY AND MATURE FRUIT COLOR

Both spine size and frequency appeared to be associated with mature fruit color. $A\chi^2$ of 13.48 and a *P* value of less than 0.01 was

obtained. Considering the number of classes involved, however, the population is so small that a small error in classification could easily change the χ^2 enough to indicate independence.

CHARACTERS APPEARING TO SHOW ASSOCIATION

In these tests for independence, the following characters appeared to show complete association: Spine size and spine frequency, spine color and fruit color, fruit netting and spine color, and fruit netting and fruit color. χ^2 values of from 141.2 to 156.0 were obtained and the P values in all cases are less than 0.01. These characters will be discussed more fully in the following paragraphs.

SPINE SIZE AND FREQUENCY

Coarse spines appear to be associated with few spines and fine spines with numerous spines. When the female parent having coarse, few spines (*FFSS*) on the fruits was crossed with the male parent having fine numerous (*ffss*) spines, a ratio of 117(*FS*):0(*Fs*):0(*fS*):39(*fs*) was obtained in the F_2 segregation. Since no plants were obtained in the coarse, numerous or fine, few spines classes, complete association is indicated between spine size and spine frequency in this cross.

SPINE COLOR AND MATURE FRUIT COLOR

Black spines appear to be associated with red and orange fruit color and white spines with yellow and cream fruit color. When the female parent having cream fruit with white spines (*ccrrbb*) was crossed with the male parent having red fruit with black spines (*CCRRBB*), a ratio of 86(*CRB*):0(*CRb*):28(*cRB*):0(*cRb*):0(*CrB*):32(*Crb*):0(*crB*):10(*crb*) was obtained in the F_2 segregation. Since no plants were obtained having red fruit and white spines, orange fruit and white spines, yellow fruit and black spines, or cream fruit and black spines, complete association is indicated between spine color and one of the two genes responsible for mature fruit color. Tkachenko (5) also concludes that white spines and white (colorless) mature fruit color are completely linked. Cochran (1) found no linkage between fruit color and spine color in an investigation in which a variety having green fruit and white spines was crossed with a variety having white fruit and black spines.

SPINE COLOR AND MATURE FRUIT NETTING

Heavy netting appears to be closely associated with black spines and no netting with white spines. When the female parent having no netting and white spines (*hhbb*) was crossed with the male parent having heavy netting and black spines (*HHBB*), a ratio of 114(*IIB*):0(*hB*):3(*Ib*):39(*hb*) was obtained in the F_2 segregation. No plants were obtained having slight or no netting and black spines and only three plants having heavy netting and white spines. Tkachenko (5) found that complete linkage exists between white spines and smooth fruit surface. These results appear to show the same thing; the small deviation that occurs in the white spine—heavy netting class possibly being due to errors in classification.

MATURE FRUIT NETTING AND MATURE FRUIT COLOR

Heavy netting also appears to be closely associated with red and orange fruit color and no netting with yellow and cream fruit color. When the female parent having no netting and cream fruit (*hhrrcc*) was crossed with the male parent having heavy netting and red fruit (*HHRCC*), a ratio of

86(*HRC*):0(*hRC*):28(*HRC*):0(*hRC*):3(*HrC*):29(*hrC*):0(*Hrc*):10(*hrc*) was obtained in the F_2 segregation. No plants were obtained having no netting and red fruit, no netting and orange fruit, heavy netting and cream fruit, and only three plants having heavy netting and yellow fruit. These results appear to indicate complete or nearly complete association between mature fruit netting and color and agree partly with the results of Tkachenko (5), who found complete linkage between white (colorless) mature fruit and smooth fruit surface. The small deviation that occurs in the heavy netting-yellow mature fruit class may be due, possibly, to errors in classification.

ASSOCIATION OF QUALITATIVE AND QUANTITATIVE CHARACTERS

In addition to the characters discussed previously a number of others were included in the study. The degree of association between these additional characters and those previously discussed is indicated in table 5, in which *t* values obtained in a comparison of the means of the phenotypic classes are presented. Observations were made on 149 to 156 individuals.

TABLE 5. Association between various characters in the F_2 progeny of a cucumber cross as indicated by the *t* values obtained in a comparison of the means of the phenotypic classes

Characters	Plant height (inches)	Number of mature fruits	Total weight of mature fruit (grams)	Weight (size) per mature fruit	Total number of fruits (mature + green)	Total weight of fruit (mature + green)	Weight per fruit (mature + green)	Days to first male flower	Days to first female flower	Number of female flowers	Number of laterals	Length per lateral	Total length of laterals
Spine color	0.06	1.24	0.30	¹ 2.39	1.07	0.60	² 2.91	1.29	1.05	0.25	1.31	1.54	1.20
Spine size	1.15	.03	1.18	² 3.34	.90	1.80	² 2.59	1.02	1.58	.25	2.34	.67	.50
Spine frequency	1.51	.03	1.18	² 3.34	.90	1.80	² 2.59	1.02	1.58	.25	2.34	.67	.50
Growth habit	² 10.22	² 18.63	² 7.26	² 4.46	² 7.29	² 7.99	² 4.49	.25	² 2.35	² 5.79	² 4.64	² 4.81	² 7.05
Mature fruit color:													
Red	.69	1.19	.25	1.56	.91	.04	1.53	.06	1.50	1.00	.68	1.09	² 3.71
Orange	1.50	1.49	.93	.64	.79	.83	.08	.91	.91	² 2.77	.17	.67	1.43
Yellow	.46	1.29	.42	.85	1.28	.30	1.08	.73	.65	.22	1.26	1.40	1.40
White	1.20	.33	1.24	² 2.76	.47	1.35	² 2.99	.99	.44	.46	.12	.24	.50
Mature fruit netting	.41	.71	.78	¹ 2.50	.42	1.13	² 2.94	1.24	.45	.25	.96	.90	.66

¹ Exceeds 0.05 point with *n* of 149. Wallace and Snedecor (6).

² Exceeds 0.01 point with *n* of 149. Wallace and Snedecor (6).

In the data presented in table 5, it can be seen that there is probably a significant difference between the determinate and indeterminate plants in all the characters presented with the exception of days to first male flower. Determinate growth habit is associated with short plant height, small number of mature fruit, small yield of mature

fruit, small weight per mature fruit, small total number of fruit (mature + green), small total weight of fruit (mature + green), small weight per fruit (mature + green), greater number of days to first female flower, short laterals, few laterals, and small total lateral length. The association between determinate growth habit and the characters pertaining to plant size may be the result of physiological causes. In other words, owing to the dwarfing effect of the determinate character, it may be impossible for determinate plants to develop the parts discussed to as great an extent as similar plants of indeterminate growth habit. Lateness of production of female flowers and small size of individual fruits may not be related to size of plant.

It is interesting to note, also, the association that is indicated in table 5 between average size per fruit, and spine color, spine size, spine frequency, fruit netting, and cream fruit color. Large fruit size appears to be associated with white spines, coarse spines, few spines, no netting, and cream fruit. As previously shown, white spines appear to be associated with cream fruit and no netting and cream fruit may be associated with no netting. If such is the case, one might expect that weight or size of fruit, when associated with one of these characters, would also be associated with the others, and, if the factors causing the expression of these characters are linked, at least one factor for size or weight of fruit would be present in the same linkage group. Furthermore, coarse spines appear to be associated with few spines but neither is associated with white spines or fruit netting and probably not with fruit color. This might indicate that the associated factors for coarse spines and few spines are in a different linkage group from the factors for spine color, fruit color, and fruit netting and that fruit size or weight appears to be associated with these characters, as well as with the group of associated characters mentioned above.

In table 5, the data also indicate a significant association between number of female flowers and orange fruit color; between number of laterals and spine size and frequency; and between total length of all laterals and red fruit color. Plants with orange-colored fruit had a smaller number of female flowers than red- yellow- or cream-fruited plants; plants with coarse, few spines had more laterals than plants with fine numerous spines; and plants with red fruit color had a greater total length of laterals than orange- or yellow-fruited plants. However, the average total lateral length per cream-fruited plant was slightly larger than that of the red-fruited plants, but owing to the fact that there were only 10 plants in this group as compared to 82 in the red-fruited group, the standard error was so high that the mean of the cream-fruited group did not differ significantly from the mean of the experiment.

INDEPENDENT ASSORTMENT OF QUANTITATIVE CHARACTERS

In a further analysis of the data obtained in this experiment, correlation studies were made in all possible combinations between characters given in table 5, on which no study of the segregation was made. Observations on 149 to 156 individuals were used to measure the association. Table 6 is a presentation of the correlation coefficients obtained in this analysis.

TABLE 6.—Correlation coefficients obtained between several plant characters in the F_2 progeny of a cucumber cross

Characters correlated	Number of mature fruits	Total weight of mature fruit	Weight (size) per mature fruit	Total number of fruits (mature+green)	Total weight of fruit (mature+green)	Weight per fruit (mature+green)	Days to first male flower	Days to first female flower	Number of female flowers	Number of laterals	Length per lateral	Total length of laterals
Plant height (inches)	1 0.74	1 0.82	1 0.51	1 0.81	1 0.86	1 0.49	0.10	2 -0.19	1 0.78	1 0.67	1 0.48	1 0.76
Number of mature fruits		1 .91	1 .24	1 .86	1 .83	1 .34	.11	1 -.22	1 .78	1 .53	1 .54	1 .72
Total weight of mature fruit			1 .56	1 .86	1 .96	1 .61	.14	2 -.20	1 .81	1 .57	1 .58	1 .79
Weight per mature fruit				1 .39	1 .63	1 .90	.12	— .04	1 .41	1 .28	1 .36	1 .41
Total number of fruits (mature+green)					1 .91	1 .29	.13	1 -.31	1 .85	1 .54	1 .61	1 .77
Total weight of fruit (mature+green)						1 .60	.14	1 -.24	1 .84	1 .57	1 .61	1 .80
Weight per fruit (mature+green)							.07	.02	1 .37	1 .28	1 .29	1 .36
Days to first male flower								— .05	.15	.03	.10	.03
Days to first female flower									1 -.27	— .01	1 -.32	1 -.20
Number of female flowers										1 .55	1 .51	1 .74
Number of laterals											.09	1 .67
Average length per lateral												1 .73

¹ Exceeds 0.01 point with n of 149. Wallace and Snedecor (6).² Exceeds 0.05 point with n of 149. Wallace and Snedecor (6).

The data presented in table 6 indicate a high degree of association between many of the characters studied. Most of these characters may be considered to be expressions of vigor of the plant, and it is interesting to note that the taller the plant is the larger are the other size or vigor characters. It will be noted also that days to the first female flower shows a significant negative correlation with all these characters, with the exception of average weight per fruit, number of laterals, and days to the first male flower.

TABLE 7.—Correlation coefficients¹ obtained between the time of production of the first male and female flower and the time of fruit maturity in 2,195 F_3 plants of a cucumber cross

Characters	Days after planting to first female flower	Days after planting to first female flower set	Days after planting to first mature fruit
Days after planting to—			
First male flower	0.0877	0.3770	0.2350
First female flower		.7604	.6719
First female flower set			.8440

¹ All highly significant (6).

There appears to be no association between number of laterals and length of lateral. Neither does days to the first male flower appear to be significantly associated with any of the other characters studied. While there appears to be a negative, although not significant, asso-

ciation between days to first male flower and days to first female flower, studies conducted on the F_3 lines in 1937 to determine the relation of time of male and female flowering to fruit maturity indicate that the relationship should be positive (table 7). The failure to obtain a significant correlation in the F_2 study may have been due to the fact that there was only a difference of about 4 days between the mean time of flowering in the parents and that the number of individuals in the F_2 population was too small to bring out the real relationship.

RELATION OF TIME OF MALE AND FEMALE FLOWERING TO TIME OF FRUIT MATURITY

A study was made with 2,195 plants from F_3 lines to determine whether time of fruit maturity, as indicated by the initiation of the change from green to the mature fruit color, could be predicted from the time of production of the first male or female flowers. The results obtained in this study are presented in table 7.

The data given in table 7 indicate a highly significant association between time of first flower production and time of fruit maturity. However, time of production of the first female flower appears more closely associated with fruit maturity than time of production of the first male flower and probably provides a better basis for prediction of earliness of maturity. While the coefficient, 0.844, indicates that the first female flower to set is the best basis for predicting earliness of maturity, it is interesting to note that there is a fairly high correlation, 0.6719, between time of production of the first female flower and earliness of maturity. This may indicate that plant selection for earliness might be made on the basis of the first female flower to appear on the plant regardless of whether it develops into a fruit or not.

SUMMARY

A study was made of inheritance in a cucumber cross between parents differing from each other in height, growth habit, spine color, spine size, spine frequency, nature fruit color, and mature fruit netting. A study was also made of the association of these and a number of other characters related to growth, flowering, and yield. The results of these studies are presented in the following outline:

1. The inheritance of the following differential characters could be explained by assuming a single factor difference.
 - a. Determinate growth habit versus indeterminate.
 - b. Black spines versus white.
 - c. Coarse spines versus fine.
 - d. Few spines versus numerous.
 - e. Mature fruit netting versus slight or no netting.
 - f. Tall plant versus short or dwarf.
2. The inheritance of mature fruit color could be explained by assuming the interaction of two independently inherited factors, the ratio obtained being approximately 9 red:3 orange:3 yellow: 1 cream.
3. The factor responsible for determinate growth also tends to decrease the height of the plant.

4. Tests for independent assortment indicate that—

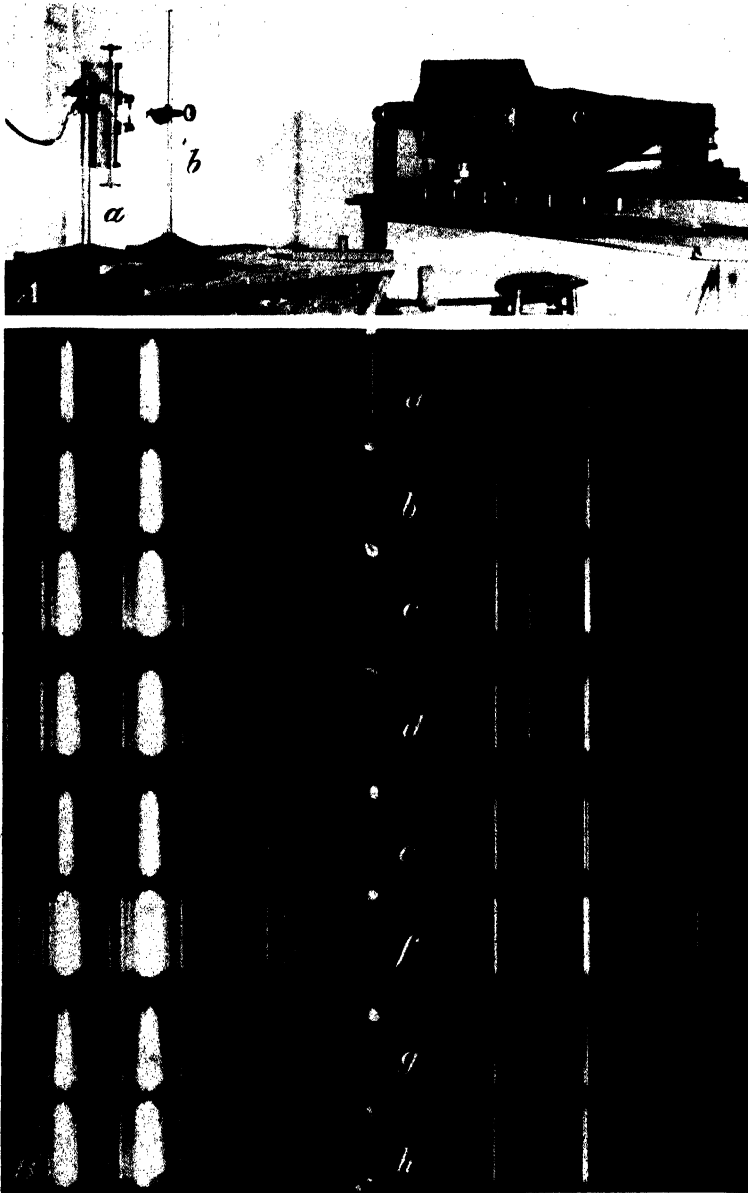
- a. The following characters appear to be associated with each other either by complete linkage or as an expression of the same factor.
 1. Coarse spines with few spines; fine spines with numerous spines.
 2. Black spines with red and orange fruit; white spines with yellow and cream.
 3. Black spines with heavy netting; white spines with no netting.
 4. Heavy netting with red and orange fruit; no netting with yellow and cream.
- b. Determinate growth habit appears to be associated with short plant, small number of mature fruit, small yield of mature fruit, small weight per mature fruit, small total number of fruit (mature+green), small total weight of fruit (mature+green), small weight per fruit (mature+green), greater number of days to first female flower, short laterals, few laterals, and small total lateral length.
- c. Large fruit size (weight) appears to be associated with white spines, coarse spines, few spines, no netting, and cream fruit.
- d. The data indicate that more than one factor may be involved in the expression of fruit size or weight.
- e. Number of female flowers appeared to be associated with fruit color; plants with orange fruit having fewer female flowers than those with red, yellow, or cream.
- f. Number of laterals appeared to be associated with spine size and frequency; plants with coarse, few spines having more laterals than plants with fine, numerous spines.
- g. In correlation studies in the F_2 progeny, plant height, number of mature fruits, total weight of mature fruit, weight (size) per mature fruit, total number of fruits (mature+green), total weight of fruit (mature+green), average weight per fruit (mature+green), number of female flowers, number of laterals, average length of lateral, and total length of laterals showed a positive and, in most cases, a highly significant association with each other when correlated in all possible combinations. Days to production of the first female flower showed a negative and, in most cases, a significant association with all the above characters. Days to the first male flower showed a positive but not significant association with the above characters.

5. A high degree of association was found between the time of the production of the first female flower by the plant and maturity of the fruit.

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A.—Equipment used in making spectrograms. *a*, Arc stand; *b*, quartz lens; and *c*, grating spectrograph.

B.—Spectrograms of tobacco ash showing region of spectrum where the 775.73 Å thallium line occurs. The dot above each spectrogram indicates the wavelength at which the thallium line should appear. *a*, Ash to which a drop of dilute thallous nitrate solution was added; *b*, ash of leaves showing severe chlorosis due to thallium toxicity; *c*, ash of leaves showing mild chlorosis; *d*, ash of leaves from plants grown in culture solution containing thallium but showing no chlorotic symptoms; *e* and *f*, soil from eastern and western Virginia on which frenching of tobacco occurred; *g* and *h*, ash of frenched leaves from peat and sand culture and from frenched plants grown in the field.

FRENCHING OF TOBACCO DISTINGUISHED FROM THALLIUM TOXICITY BY SPECTROGRAPHIC ANALYSIS¹

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INTRODUCTION

Several theories as to the cause of tobacco frenching have been proposed, but each in its turn has been found to be erroneous. Recently, it has been postulated that frenching is probably a symptom of thallium toxicity. Since the demonstration of the presence of thallium in frenched tobacco tissue in toxic amounts or its absence is essential to an evaluation of the thallium-toxicity theory, and the quantity of thallium necessary to produce thallium toxicity under experimental conditions is too minute to be detected by ordinary chemical methods, this theory at first seemed impossible to prove or disprove. However, since spectrographic analysis has been shown by a number of investigators (9, 10, 16)² to be applicable to the determination of minute quantities of minerals in plant tissues, the spectrographic method seemed to be applicable to the evaluation of the thallium-toxicity theory. Accordingly, a cooperative study was undertaken in 1937 between the Virginia Agricultural Experiment Station and the Physics Department of the Virginia Polytechnic Institute to determine by spectrographic analysis whether there is any relationship between frenching and thallium toxicity of tobacco. Preliminary reports on this study have been made by the authors.³

REVIEW OF LITERATURE

Results reported by Shear (11) in 1933 led him to propose a toxicity theory to explain the cause of frenching, although the nature of the toxin was not determined. Karraker and Bortner (6), although they state that they do not think frenching is of toxic origin, present no evidence that is incompatible with such a theory, and the following quotations from the summary of their work, published in 1934, are most easily explainable by such a theory (6, p. 108).

Frenching in plants in soil and river sand in the greenhouse was rather easily brought about by proper addition of pulverized limestone and adjustment of

¹ Received for publication October 2, 1939. Paper No. 106 of the Section of Botany and Plant Pathology, Virginia Agricultural Experiment Station. This paper was awarded the Jefferson medal of the Virginia Academy of Science at the seventeenth annual meeting, May 5, 1939, Danville, Va. This project was partly financed by a research grant from the Virginia Academy of Science.

² Italic numbers in parentheses refer to Literature Cited, p. 139.

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nutrient supply. It was more difficult to obtain frencing when acid-treated and washed white silica sand was used.

* * * * *
While the results of the studies rather clearly show frencing to be related to soil reaction and nutrient supply as described, yet for various reasons it can not be concluded that other causal factors are not present with which or thru which the reaction and nutrient factors may function. And it may be, even, that reaction and nutrient factors merely suppress or bring into activity some other factor which is the underlying cause, somewhat as moisture supply affects frencing by affecting rate of growth.

The treatment of the sand referred to above should not add anything to the sand but should tend to remove most soluble material contained in it. If, thereby, the tendency toward frencing is reduced the washing must have removed something from the sand which was toxic to the tobacco. If, as the authors suggest, the combination of soil reaction and nutrient supply may bring into activity some other factor which is the underlying cause, then that factor must be a toxic substance of some kind.

In 1935 Spencer (12) reached the conclusion that frencing was not a mineral-deficiency disease but was a toxicity disease. At that time he proposed no explanation as to the cause of the toxic effect.

Thallium toxicity was first compared to frencing of tobacco by McMurtrey in 1932 (8). He says:

Whether the typical frencing disease of tobacco is due to thallium toxicity cannot be definitely stated at this time, but it appears that there is much in common in growth manifestations exhibited by the two pathological conditions.

Spencer (13) made an extensive study of frencing and thallium toxicity and showed that in most respects the symptoms of the two are the same. He found that different species of tobacco each showed the same susceptibility or resistance to both frencing and thallium toxicity, and that both disorders were completely controlled by applications of available nitrogen, aluminum, or potassium iodide, and partly controlled by steam sterilization. He found that smaller applications of thallium salts were required to produce a toxic effect on tobacco grown on soil that caused frencing than on nonfrenching soils. He concludes his paper with the following statement:

The experimental evidence reported indicates a striking similarity between natural frencing and thallium toxicity not only with respect to the symptoms they produce on Turkish tobacco but also with regard to the methods by which they are controlled. Final proof that these two diseases are identical requires the demonstration of the presence of toxic quantities of thallium in soils which produce frencing. At present it is questionable whether the known chemical methods are sufficiently sensitive to detect such small traces of thallium as the experiments here reported have indicated must be present in the soil if frencing is a thallium-toxicity disease.

The following statement appearing in Connecticut Experiment Station Bulletin 410 (1, p. 406), further emphasizes the difficult nature of this problem:

The real difficulty, however, in identifying the two maladies, lies in the fact that there are no known methods of measuring and comparing the amounts of thallium contained in frenched tobacco leaves and in those known to be injured by thallium.

After the presentation of the paper by Shear and Ussery⁴ in December 1938, in which they presented positive spectroscopic evidence to

⁴ See footnote 3.

show that the two disorders are not the same, Spencer and Lavin (14) published a brief article based on very limited spectroscopic data in which they concede that frenching and thallium toxicity may not be the same.

METHODS OF EXPERIMENTATION

The tobacco used in this study consisted of varieties of *Nicotiana tabacum* L.

The plants receiving thallium as thallium nitrate were grown in water culture. Six or eight plants were grown in each treatment, and representative material from each plant in a treatment was collected for each analysis. The standard nutrient solution used made available the following amounts of elements per plant:

Element:	Milligrams
Nitrogen.....	406
Phosphorus.....	56
Potassium.....	487
Calcium.....	316
Magnesium.....	40
Chlorine.....	48
Sulfur.....	40
Sodium.....	30
Manganese.....	1. 2
Iron.....	. 99
Boron.....	. 85

For the lower concentrations of nutrients, dilutions of this formula were used.

The plant tissue to be analyzed was dried in an oven and then ashed at a dull red heat in porcelain crucibles over a gas burner. Twenty-four milligrams (± 0.5 mg.) of ash was packed into the hollowed end of the lower + electrode for analysis.

The electrodes were made from No. 3 B. & S. gage copper wire. The ends of the electrodes were cut down on a lathe to 4 mm. in diameter. The diameter of the hole in the lower electrodes was 3.5 mm.

A grating spectrograph⁵ with a dispersion of 9.43 Å. per millimeter in the first order and a resolving power of 25,000 was used in making this study. The spectrograph and arc stand are shown in plate 1, A. The arc was operated by a 120-volt direct current generator, the current used varying from 8 to 10 a. The arc was focused on the slit by means of a quartz lens. An exposure of 30 seconds was used for all the plant ash samples herein reported.

As a densitometer was not available for making quantitative determinations a direct visual comparison was made by the method of homologous pairs which Gerlach and Schweitzer (2) used in evaluating spark spectra. Various amounts of thallium were added to samples of tobacco ash that showed no thallium. Seventy-two milligrams of such ash was wet with enough thallium nitrate solution to give the desired percentages of thallium. After being thoroughly dried and mixed, 24-mg. samples were used in making the determinations. On each spectrogram of an ash containing a known amount of thallium a copper line of equal density and as near the thallium line as possible was selected and used as a basis for evaluating the spectrograms of the ash samples.

⁵ Grating ruled by Dr. J. A. Anderson.

When the density of the line at 3,779.3 Å. matched that of the 3,775.7-Å. Tl line the ash contained no thallium but the exposure had been long enough to cause the thallium in the copper to appear. If the 3,775.7-Å. Tl line matched the 3,780.0-Å. Cu line then the ash contained less than 0.0001 percent of thallium. When the 3,775.7-Å. Tl line matched the 3,800.4-Å. Cu line the ash contained 0.0002 percent of thallium. When the 3,775.7-Å. Tl line matched the 3,759.5-Å. Cu line the ash contained 0.0004 percent of thallium. When the 3,775.7-Å. Tl line matched the 3,771.9-Å. Cu line the ash contained 0.0006 percent of thallium. When the 3,775.7-Å. Tl line matched the 3,741.2-Å. Cu line the ash contained 0.001 percent of thallium. When the 3,519.2-Å. Tl line matched the 3,520.0-Å. Cu line the ash contained 0.005 percent of thallium. In these spectrograms, the criterion for proper exposure was that the pair of copper lines 3,450.33 Å. and 3,457.85 Å. were a visual match in density, since these lines were found to vary in relative intensity with time of exposure and conditions of excitation. The wave-length values used were those listed by Kayser (7).

EXPERIMENTAL DATA

During the course of this study, approximately 200 spectrograms were made, about half of which were of ash samples. The original plates were used exclusively in determining the amount, if any, of thallium present in the ash samples analyzed.

The first step in this study of the tobacco ash samples was to determine whether thallium could be detected in the ash of healthy and frenched tobacco grown on different soils. Six samples of frenched leaves were analyzed. Four of these were from field-grown plants collected in southwest, central, and eastern Virginia, and from central North Carolina. Two of the frenched samples came from plants grown in the greenhouse, one in soil and one in a peat-sand mixture. Healthy leaves from field-grown plants, from plants grown in frenching soil that had been steamed, and from plants grown in culture solutions receiving no thallium were used. Two soils which caused frenching of tobacco were also analyzed. None of these samples showed any indication of thallium in the spectrograms. Several representative spectrograms are shown in plate 1, B.

To determine whether or not thallium could be detected in the ash of plants which had received small amounts of this substance, tobacco plants were grown in nutrient solution to which thallium nitrate was added. Two sets of eight plants each were grown in full strength nutrient solution for 1 week and then thallium was added at the rate of 0.10 p. p. m. to one set and 0.05 p. p. m. to the other set. The nutrient solution was not renewed but additional thallium was added at weekly intervals for 3 weeks, when chlorosis due to thallium toxicity appeared on some of the plants. At this time leaf samples were collected for analysis. Three samples from the plants receiving thallium were taken, one of the leaves showing severe chlorosis, one of the leaves showing mild chlorosis, and one of the leaves showing no chlorosis. On analysis the ash of these samples gave 0.0006, 0.0003,

and 0.0002 percent of thallium respectively, thus showing a relationship between the severity of the symptoms and the amount of thallium in the tissue.

Spencer, in discussing these results with the senior author on December 29, 1938, stated that the results might not be conclusive in showing the two disorders to be distinct since only one concentration of nutrient solution was used. His results had indicated that the amount of thallium required to cause toxic symptoms decreased with a decrease in nutrient content of the solution in which the plants were grown; hence, there was a possibility that the nutrient solution could be diluted to a point where the thallium required to produce toxic symptoms could not be detected in the leaf ash by spectrographic analysis.

A series of experiments was therefore conducted in which the nutrient solution and the amount of thallium added were varied to determine their effect on the appearance of chlorosis and the percentage of thallium in the ash. The results obtained from the first sets of plants are shown in tables 1 and 2. Plants with stems 1 to 2 inches long were grown 1 week in standard-strength nutrient solution. Twelve plants were then placed in each of the following solutions: Standard strength, two third standard strength, and one third standard strength. Half of the plants in each of these groups received 0.067 p. p. m. of thallium, while the other half received 0.033 p. p. m. No further treatment was given these plants except for aerating the solution twice daily and maintaining the solution at a constant level by adding distilled water. The plants were growing rapidly when the thallium was added and, with the exception of those in the weakest nutrient solution, there was no noticeable difference in growth or appearance of plants. The plants in the one third strength nutrient solution did not grow so rapidly as the others and after about 2 weeks the young leaves developed a diffuse chlorosis not typical of the chlorosis produced by thallium.

TABLE 1.—*Thallium in tobacco ash from young leaves of tobacco grown in culture solutions of varying nutrient and thallium content; samples taken 1 and 2 weeks after addition of thallium*

Concentration of nutrient solution	1 week after addition of thallium		2 weeks after addition of thallium	
	Concentration of thallium in nutrient solution	Concentration of thallium in leaf ash	Concentration of thallium in nutrient solution	Concentration of thallium in leaf ash
	P. p. m.	Percent	P. p. m.	Percent
Standard.....	0.067	0.0001	0.067	0.0001
Do.....	.033	(1)	.033	(1)
Two-thirds standard.....	.067	(1)	.067	.0001
Do.....	.033	(1)	.033	(1)
One-third standard.....	.067	(1)	.067	.0006
Do.....	.033	(2)	.033	.0003
From soil.....			(1)	(2)

¹ Present but less than 0.0001 percent.

² Presence doubtful.

³ None.

TABLE 2.—*Thallium in tobacco ash from different parts of tobacco plants grown in one-third standard strength culture solutions of varying thallium content; samples taken 3 weeks after addition of thallium*

Portion of plant used	Concentration of thallium in nutrient solution	Concentration of thallium in ash of root, stem, or leaf	Portion of plant used	Concentration of thallium in nutrient solution	Concentration of thallium in ash of root, stem, or leaf
	<i>P. p. m.</i>	<i>Percent</i>		<i>P. p. m.</i>	<i>Percent</i>
Roots	0.067	0.005	Lower leaves	0.067	0.001
Do033	.002	Do033	.0002
Stems067	.0003	Upper leaves067	.0001
Do033	.0002	Do033	(¹)

¹ Present but less than 0.0001 percent.

Samples consisting of young leaves from each treatment were collected 1 week and 2 weeks after the thallium was added. Each sample consisted of leaves from all the plants receiving the same treatment. The first set of samples showed the greatest amount of thallium in the leaves of plants grown in standard strength nutrient solution plus 0.067 p.p.m. of thallium. The presence of thallium was doubtful in the sample from plants in one-third standard strength nutrient solution plus 0.033 p. p. m. of thallium. All the samples between these extremes showed essentially the same amount of thallium. By the end of the second week this relationship had changed very markedly. The plants receiving the larger amount of thallium showed a higher percentage of thallium than the plants receiving the same strength of nutrient solution but a smaller amount of thallium. The thallium in the ash of plants in the one-third strength nutrient solution receiving 0.067 p. p. m. and in the one-third strength nutrient solution receiving 0.033 p. p. m. of thallium had reached 0.0006 and 0.0003 p. p. m., respectively.

After the plants had grown for 3 weeks in the thallium solutions the experiment was discontinued as the plants were becoming crowded. The two sets of plants in the one-third strength nutrient solution were separated for analysis into root, stem, old leaf, and young leaf tissue. The results of these analyses are shown in table 2. It may be seen from these results that the greatest amount of thallium is found in the roots, with an intermediate amount in the stems and older leaves and the smallest amount in the young leaves. In each case the ash of plants receiving the least thallium gave a smaller percentage of this material than was found in the ash of the same part of the other plants.

When the next series of plants was harvested, the six plants in the one-third strength nutrient solution receiving 0.033 p. p. m. of thallium were divided for analysis into lower half of root system, upper half of root system, lower part of stem, old leaves, and young leaves. Although these plants had been in the nutrient solution twice as long as the previous set the plants were about the same size because they were grown in distilled water for several days before the nutrient solution was added, which slowed down their growth rate.

The results of these analyses were as follows:

Part of plant used: ¹	Concentration of thallium in ash, percent
Lower half of root system----	0.0002
Upper half of root system----	Between 0.0006 and 0.0008
Stems-----	Present but less than 0.0001; more than in old leaves
Old leaves-----	Present but less than 0.0001; more than in young leaves
Young leaves-----	Present but less than 0.0001

¹ Samples taken 6 weeks after addition of thallium.

These results substantiate those of the previous analyses in that they show a marked gradient in thallium decreasing from the roots to the youngest leaves.

It will be noted that the percentage of thallium in the ash of the young leaves had significantly decreased from the second to the third week after treatment. Since no marked toxic effects were observed in the first sets of dilutions of nutrient solution, other sets were prepared in which still more dilute solutions with different amounts of thallium were used. In order to reduce the nutrient reserve in these plants they were grown in distilled water for 4 days before nutrients and thallium were added. This experiment included groups of plants receiving one-third strength, one-sixth strength, and one-twelfth strength nutrient solution, half of the plants in each group receiving in addition 0.067 p. p. m. of thallium and the other half 0.033 p. p. m. The results of this experiment are shown in table 3. The plants in these groups grew very slowly and all showed a marked toxic effect on their roots. The roots stopped growing as soon as they penetrated the solution and a mat of short roots formed just above the surface of the surface of the solution. After 2 weeks the roots of the plants in the one-third strength nutrient solution with 0.033 p. p. m. of thallium were able to grow in the solution without further injury, and the severity of the root injury was reduced somewhat in the one-third strength nutrient solution with the larger amount of thallium. The roots of the other plants showed no indications of recovery after 4 weeks in the solutions. Samples were collected from these plants 2½ weeks and 4 weeks after treatment. The spectrograms of the first samples could not be used as the equipment was not adjusted properly; and since there was originally only a small amount of material available, there was not sufficient ash to repeat the analyses on this set of samples. The results shown are from the second set of samples. All of these samples showed measurable amounts of thallium but the differences between the samples were not great.

TABLE 3.—*Thallium in tobacco ash from young leaves of tobacco grown in culture solutions of different nutrient and thallium content*

Concentration of nutrient solution	Concentration of thallium in nutrient solution	Concentration of thallium in ash	Concentration of nutrient solution	Concentration of thallium in nutrient solution	Concentration of thallium in ash
	P. p. m.	Percent		P. p. m.	Percent
One-third standard-----	10.067	0.0003	One-twelfth standard-----	10.333	0.0002
Do-----	1.033	.0003	Do-----	2.016	(2)
One-sixth standard-----	1.067	.0002	Do-----	2.008	(4)
Do-----	1.033	.0002-	Do-----	2.000	(4)
One-twelfth standard-----	1.067	.0003			

¹ Samples collected 4 weeks after addition of nutrients and thallium.

² Samples collected 1 week after addition of nutrients and thallium.

³ Present but less than 0.0001 percent.

⁴ None.

After the plants had grown in the thallium solutions for 3 weeks, typical thallium-induced chlorosis appeared on leaves of plants in three different treatments. Five plants out of six in the one-sixth strength nutrient solution plus 0.067 p. p. m. of thallium showed mild chlorosis, but by the end of the fourth week the youngest leaves appeared healthy. Three plants out of six in the one-twelfth strength nutrient solution plus 0.067 p. p. m. of thallium developed chlorosis which showed up on the youngest leaves at the end of 5 weeks. Two plants in the one-twelfth strength nutrient solution plus 0.033 p. p. m. of thallium developed a mild chlorosis which no longer appeared on the youngest leaves after 4 weeks.

With another group of plants in one-twelfth strength nutrient solution the thallium content was reduced to 0.016 and 0.008 p. p. m. (table 3), and after 1 week a trace of thallium could be detected in the leaves of plants receiving 0.016 p. p. m. of thallium but none could be detected in the plants receiving the smaller amount.

DISCUSSION

It might be argued that by further manipulation of the nutrient solution, such as an alteration in the ratio of the various nutrients, leaf chlorosis might be produced with a smaller amount of thallium available to the plant, but such an assumption would not seem plausible on the basis of the experiments reported here. The ratio of the nutrients used in the nutrient solutions employed in these studies was based on a number of analyses of healthy field-grown tobacco and therefore the ratio of the various elements should be nearly optimum. It would, therefore, appear that no one element could be significantly reduced without a corresponding reduction in growth. The nutrient level was reduced in this study until very little growth was produced, and yet thallium in too small an amount to cause chlorosis could be detected. Since in most cases of frencing growth is more rapid than this, thallium, if the causal factor, would be present in measureable quantities. In this study the smallest amount of thallium added to the nutrient solution that could be detected in the plant ash was only one-fourth the minimal amount required in culture solution to cause chlorosis, according to Spencer (13).

The results show a gradient in thallium in the tobacco plant, decreasing from the roots to the tip of the shoot. This gradient existing several weeks after the addition of the thallium indicates that it is deposited in an immobile state in the plant. It might be precipitated in an organic or an inorganic compound. Since the thallium is not precipitated in the nutrient solution its precipitation in the plant as an inorganic salt would have to be brought about by some chemical change effected by the metabolism of the plant. Whatever the compound in which the thallium is fixed, its components or catalyzer must be present in extremely minute quantity; otherwise with the small amount of thallium available all of it should be precipitated in the roots. The fact that a gradient occurs would support such an hypothesis.

Since the results of these experiments are so conclusive in showing that frencing is not thallium toxicity, it might be well to point out facts from the results of other investigators that could not be fitted into the frencing-thallium toxicity theory. In the first place

the symptoms of the two disorders are not identical, as was pointed out by McMurtrey (8). In the case of thallium toxicity, the first indications of chlorosis are along the midrib or the leaf; whereas in frenching, chlorosis starts at the borders of the leaf. Spencer (12) showed that 1 part of frenching soil in 2,000 parts of washed quartz sand was sufficient to cause frenching. He has also shown that it takes approximately 0.10 p. p. m. of thallium in sand to cause thallium-toxicity symptoms to appear on tobacco grown in the sand. If these two disorders were the same, then the frenching soil would contain about 400 pounds of soluble thallium per acre or 200 p. p. m., which is 800 times as much as was required to produce thallium toxicity symptoms on tobacco in one of the soils with which he worked. His finding, that less thallium was required to produce toxic symptoms on tobacco growing in frenching soil than in other soil, does not necessarily mean that the thallium which he added supplements some already in the frenching soil; it might just as readily mean that the absorptive power of such a soil was low and therefore neither the thallium nor the frenching toxin was fixed as they might be in a soil that would not produce frenching.

Steinberg (15, p. 574), in a recent paper on the essentiality of gallium to growth and reproduction of *Aspergillus niger*, makes the following statement:

The reported toxicity of thallium, a chemical homologue of gallium, to tobacco by McMurtrey, in association with the close duplication of the symptoms of frenching, would appear to indicate the possibility that thallium prevented the utilization of gallium by the plant.

He draws the analogy on the basis of Hurd-Karrer's work in which she shows that the toxic effects of selenium, arsenic, rubidium, and strontium can be prevented by sulfur, phosphorus, potassium, and calcium respectively. She concludes from these findings that the salt of a toxic element can be rendered nontoxic to plants through the addition of a sufficient amount of the salt of an essential element in the same chemical group. She clearly shows that the toxic action is not due to the injurious element preventing the absorption or utilization of the essential element, which is just the reverse of the assumption upon which Steinberg appears to base his hypothesis. It is entirely possible on the basis of Hurd-Karrer's results (3, 4, 5) that gallium might, if present in sufficient amount, counteract thallium toxicity, although its absence could not be construed as the cause of the symptoms produced by thallium.

An examination of the spectrograms in the region of the persistent gallium lines at 4,033.01 and 4,172.05 Å. revealed no indications of gallium in any of the ash samples. The chemical properties of gallium are such that it would be more easily excited in the arc than thallium and therefore should be detectable in more minute amounts than thallium. Thus if gallium is essential for the growth of tobacco the amount necessary must be less than 0.0001 percent in the ash.

The theory that frenching is caused by an unstable organic toxin which accumulates only under certain abnormal conditions and which is produced by some widely distributed soil organism is herewith proposed. This theory is based on a number of facts which have been discovered concerning the conditions under which frenching develops. Frenching has been reported from most regions in the

world where tobacco has been grown, proving the wide distribution of the underlying cause. Toxic minerals which might conceivably cause frenching are not so universally distributed, whereas certain types of organisms are. Partial sterilization of the soil temporarily renders it incapable of producing frenching. The instability of the toxic factor has been shown by several experiments. A frenched plant recovers rapidly when the ball of soil in which it is growing is tapped from the pot and lightly replaced. Frenching could be produced in sand cultures watered with the leachate of frenching soil when the leachate was conducted from the soil to the sand through a separatory funnel. When the soil and water were mixed and the supernatant liquid used for watering sand cultures no frenching developed.

Frenching cannot be produced readily on tobacco in water cultures. Under such conditions very few normal soil organisms can grow. Frenching has been produced on tobacco in sand cultures under various conditions. Spencer, for example, obtained frenching on tobacco seedlings growing in sand containing 1 part of frenching soil in 2,000 parts of sand, a result which supports the organic-toxin theory, since the influence of such a small amount of soil could be explained most logically on the basis of its being an inoculum of soil organisms. He found that frenching could be produced in plain sand if tobacco were grown in the sand for a sufficient time. This might be explained on the basis of there being insufficient organic matter in the sand until some dead tobacco roots accumulated.

SUMMARY AND CONCLUSIONS

A spectrographic analysis of the ash of tobacco samples from healthy, frenched, and thallium-treated plants was made. Samples of soil in which frenched tobacco had occurred were also analyzed.

Thallium could be detected only in samples of ash from plants grown in a nutrient solution to which thallium had been added, proving that frenching and thallium toxicity are not the same. Thallium could be detected in ash samples in amounts of less than 0.0001 percent and in ash samples from plants receiving only 0.016 of a part per million of thallium in the nutrient solution.

The weaker the nutrient solution the smaller the amount of thallium necessary to produce toxic symptoms.

Thallium is fixed in the plant tissue in such a way as to produce a gradient, decreasing from the roots to the youngest leaves. The plant, therefore, must have a continuous supply of available thallium if thallium toxicity symptoms are to continue to appear on the new growth as it is produced.

Gallium does not appear to have any relationship to frenching or thallium toxicity.

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THE VITAMIN A ACTIVITY AND THE VITAMIN B₁ CONTENT OF SOYBEANS AND COWPEAS¹

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INTRODUCTION

In view of the wide use of soybeans (*Soja max*) and cowpeas (*Vigna sinensis*) as food for both man and animals, and the relative scarcity of data concerning their vitamin A activity and vitamin B₁ content, it seems desirable to publish the results of assays made on several varieties of these leguminous seeds.

The available results of vitamin assays of these seeds have been compiled by Daniel and Munsell (1)² and by Fixen and Roscoe (2). They are summarized in table 1.

TABLE 1.—Carotene and vitamin A and B₁ content of soybeans and cowpeas as reported by various workers

Product	Carotene per gram	Vitamin A activity per gram	Vitamin B ₁	
			Per gram	Per gram
	Micrograms	Sherman units	Sherman units	International units
Soybean (<i>Soja max</i>):				
Dried ¹			1.4	
Yellow ¹			7.7	
Mammoth Yellow ¹			4.0	
Soya bean (<i>Glycine soja</i>) ²	4.5-9.7			1.0, 1.5
Yellow ²				<0.45
Black ²				1.0
Soybean ³	0.4-0.8			
Mammoth yellow ⁴	.50			
Tokio ⁴	.49			
Tarheel black ⁴	.38			
Biloxi ⁴	.37			
Rokusun ⁴	.29, .38			
36 other varieties ⁴	.17-2.44			
Cowpea (<i>Vigna sinensis</i>) ²				1.7
Dried ¹		2.0		
Black-eyed ¹		3.0		
Cream Crowder ⁴	.33, .25			
Brown Crowder ⁴	.26			
Virginia blackeye ⁴	.27			
5 other varieties ⁴	.26-.33			

¹ Data from Daniel and Munsell (1).

² Data from Fixen and Roscoe (2).

³ Data from Lanzig and Van Veen (3).

⁴ Data from Sherman and Salmon (6).

EXPERIMENTAL PROCEDURE

Vitamin B₁ was determined by the method described by Sherwood and Halverson (7).

The basal ration for vitamin A assays consisted of purified casein 18 percent, cornstarch 54 percent, irradiated dried yeast 7.5 percent, Wesson salt mixture (8) 3.5 percent, agar-agar 2 percent, and hydrogenated vegetable fat 15 percent.

¹ Received for publication October 16, 1939. Paper No. 109 of the Technical Series, North Carolina Agricultural Experiment Station.

² Italic numbers in parentheses refer to Literature Cited, p. 143.

The mothers of the rats used for the assays received Sherman's diet B as modified by Russell (5) until the litters were 12 to 14 days old, when they were changed to the above-described vitamin-A-free basal ration. After weaning, the young rats were kept on the basal ration until they ceased to gain in weight. Almost invariably at this time, about 35 days after weaning, ophthalmia was moderately severe. The rats were then placed in individual screen-bottomed cages, continued on the basal ration, and one-half of them were given daily 1 gm. of the sample being tested. The remaining animals were kept as negative controls.

The sample is reported as free from vitamin-A activity when the test rats, usually 4 for each sample continued to lose weight and died in about the same length of time as their controls. Those samples that promoted growth in the test rats and alleviated the ophthalmia were assayed quantitatively. Usually each of 10 rats was fed daily 1 gm. of the sample being assayed, and at the same time an equal number of rats were given daily definite doses (at least 2 planes) of the International Standard carotene dissolved in refined cottonseed oil (Wesson oil). All rats received the basal ration *ad libitum*. The standard carotene solution was sealed in glass ampoules under nitrogen gas as soon as made and kept in the freezing compartment of an electric refrigerator until needed. After an ampoule was opened its contents (enough for 3 to 7 days) were kept in the refrigerator when not in actual use.

One sample of Tokyo soybeans was grown on the experiment station farm and one of Rokusun soybeans was obtained through the courtesy of Charles Dearing from the Coastal Plain test farm. All others were obtained from a local seed dealer.

All samples were of high viability³ and were carefully hand picked, only seed of uniform color and size being retained for the assays. The samples were kept in the refrigerator until assayed.

RESULTS OF ASSAYS

The results of the assays are given in table 2.

TABLE 2.—*Vitamin A and B₁ content of different varieties of soybeans and cowpea*

Variety	Vitamin A per gram	Vitamin B ₁ per gram	Variety	Vitamin A per gram	Vitamin B ₁ per gram
	<i>International units</i>	<i>International units</i>		<i>International units</i>	<i>International units</i>
Soybeans:			Cowpeas:		
Laredo.....	1.3	4.1	Whippoorwill.....	0	2.3
Herman.....	.5	3.6	Brabham.....	0	2.7
Tokyo.....	0	3.2	Groft.....	.5	3.0
Do.....	.5	3.2	Blackeye.....	0	3.5
Biloxi.....	0	3.9	Iron.....	0	3.5
Do.....	0	3.5	Clay.....	0	3.7
Mammoth Yellow.....	0	3.8	Sugar Crowder (cream).....	0	2.6
Do.....	0	3.2	Sugar Crowder (brown).....	.5	2.6
Otootan.....	1.3	3.7	Small Black.....	0	2.8
Do.....	.5	4.1	Do.....	0	3.1
Virginia.....	0	4.8			
Do.....	.5	4.3			
Tarheel Black.....	.5	3.3			
Do.....	0	4.1			
Rokusun.....	0	3.9			
			Average.....		3.0
Average.....		3.8			

³ Tested through the courtesy of J. W. Woodside of the seed laboratory of the State Department of Agriculture.

The results of the biological tests for vitamin-A activity of soybeans are in accord with those of animal feeders, who find that soybeans "supply no appreciable amount of vitamin A" (4, p. 370).

The quantities of carotene in the named varieties of soybeans and cowpeas in table 1 are equivalent to 0.4 to 0.8 International Unit of vitamin A per gram. These figures do not differ significantly from those in table 2 when it is considered that the carotene assay is capable of evaluating much smaller quantities than the biological method and that the amounts present are at or below the lower limit of the latter. Some difference between the two sets of assays is to be expected because of seasonal and other variations within the varieties, because of differences in maturity when harvested (9), and because all of the carotene present may not be utilized by the animals (9).

The varieties of soybeans assayed contained somewhat more vitamin B₁ than the cowpeas, although the differences are not great, and some samples of cowpeas were as rich in this factor as some of the soybeans. The values in table 2 are two to four times as great as those previously reported (table 1). The data given in table 1, however, are too meager to warrant any detailed comparisons. Both soybeans and cowpeas may be regarded as excellent sources of vitamin B₁.

SUMMARY

Nine common varieties of soybeans and eight of cowpeas, 15 and 10 samples respectively, were purchased from a seed store and assayed by the rat-growth method for their vitamin-A activity and vitamin-B₁ content.

The results show that none of these varieties of soybeans or cowpeas contain appreciable quantities of vitamin A. The soybeans contain 3.2 to 4.8 International Units of vitamin B₁ per gram and the cowpeas 2.3 to 3.7. There are no marked differences between varieties.

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THE DETERIORATION OF THE BOVINE UDDER IN THE ABSENCE OF STREPTOCOCCI¹

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INTRODUCTION

The rate of decline in the productive life of the modern dairy cow approximates 25 percent annually in the chief dairy regions of the United States and reaches 50 percent in large herds maintained by purchase. Many observers class deterioration of the udder as one of the important if not the most important factor in such "wastage" of dairy cows.

The role which certain of the streptococci play in the deterioration of the mammary gland of the cow has, during the past decade, been studied in each of the important dairy regions of the world. These bacteria are unanimously considered as the most important agency in the causation of udder troubles.

The significance of other bacteria, such as certain of the staphylococci, has recently been emphasized, and the literature of this phase of the question has been presented by Plastring and his associates, as have the extended observations made by them (11).²

The possibility that there may be agents other than bacteria causing deterioration of the mammary gland has been suggested by Meigs and his associates (7) who studied the effect of milking machines in producing such changes in the milk as are indicative of chronic inflammation of the gland.

A filtratable virus was isolated from cows having mastitis by Broadhurst and her coworkers (1). Hastings and Beach (3, 4) described cases of chronic mastitis in a herd of cows free from udder streptococci. The phrase "nonspecific mastitis" was used to indicate that no specific cause was found for the disturbances noted. The observations presented in these and in other papers indicate the complexity of the subject and emphasize the need of more extended observations made under a wide variety of conditions.

The present paper reports the observations made on a group of Holstein-Friesian heifers assembled previous to their first lactation period for the purpose of studying certain aspects of nutrition.

THE HERD AND THE METHODS

The 18 animals used in this experiment were kept in stable and yard constantly, and each received a ration which was not below that used on many dairy farms either from the quantitative or qualitative point of view. It thus seems that the abnormalities noted in the function and in the tissues of the udder cannot have been due to deficiencies in the feed.

¹ Received for publication November 20, 1939.

² Italic numbers in parentheses refer to Literature Cited, p. 155.

The cows were milked by a machine with care as to vacuum used and the length of time the machines were left on the cows. On 1 day of each week each cow was milked, morning and evening, by the machine so modified as to enable one to determine the yield of each separate quarter. The samples to be used for chemical determinations consisted of the first 30 cc. of milk from each quarter drawn into separate bottles, while those for bacteriological examination consisted of a few cubic centimeters drawn into a sterile container immediately after the milk for the chemical tests had been taken. The chlorine in the chlorides of the milk was determined by titration with silver nitrate, 10 cc. of milk diluted with 80 cc. of water in the presence of 2 cc. of a 10-percent solution of potassium dichromate being used. It is recognized that this method gives higher apparent values than that employed in previous studies (3, 4). Since consideration of the data is confined to the group of animals observed, this limitation is of no significance.

The values given for catalase represent the volume of oxygen evolved from a mixture of 2 volumes of the sample and 1 volume of a 1-percent solution of hydrogen peroxide, expressed in percentage of the volume of milk used in the test. The method devised by the senior author for collecting and measuring the gas has been briefly described in a previous paper (5). Owing to the content of all body cells in catalase and to the relative, if not absolute, freedom of such fluids as the blood serum therefrom, the catalase values are believed to be an indirect measure of the content of the milk in intact body cells of all types and in cellular debris. The catalase value seems to have all the significance that an enumeration of cells has and is obtained with less effort.

CHLORINE AND LACTOSE

Davies (2) has shown that the chlorine content of the milk of different breeds is not the same, and it may be that the differences between individual animals of the same breed are significant. The observations of Davies show the fallacy of using any value such as 0.14 percent as an indication of disturbance in the udder when nothing is known about the normal level of chlorine in the milk of the animal in question. It seems safe to assume that the values for chlorine and catalase found in the milk produced by an animal in that part of the first lactation period immediately after the colostric stage, represent the base levels for that animal, and that persistent and marked deviations therefrom indicate a deterioration of the udder, and especially of individual quarters which show more marked deviations from the base level than other quarters, particularly when such changes are accompanied by a decreased quantity of milk. It is generally believed that the milk is isotonic with the blood, the freezing point of which is supposed to remain constant. The lactose and chlorides are believed to be the principal agents in governing the freezing point of milk, hence it follows that an increase in chlorine of 1 unit will necessitate a decrease in lactose of 11.1 units (12). A decrease in the percentage of lactose indicates a profound change in the procedure of milk synthesis in the udder.

In previous studies, in the absence of more definite reference points for chlorine and catalase values, use was made of arbitrary

values to separate the normal from the abnormal; thus 0.15 percent of chlorine was the upper normal limit for chlorine, and 50 the maximum value for normal catalase.

In the present studies more definite reference points have been available since the observations on the composition of the milk began at the beginning of the first lactation. It is assumed that the values found in the first 60-day interval after calving represent the base level of the animal in question, an assumption made more certain when the values of all quarters are much the same.

THE FINDINGS

BACTERIOLOGICAL FINDINGS

At the beginning of the study the bacteriological methods employed were those commonly used for the detection of streptococci. It soon became evident that this group of cows, like the one formerly studied, was free from udder streptococci, a condition which continued throughout the period of observation. Some students of chronic mastitis believe that streptococci are present in the mammary gland of every cow, and that failure to demonstrate them is owing to procedures used. It would seem that had streptococci been present in any animal of the group, they would have reached easily detectable numbers in some one of the animals at some time in the period of observation, if they were responsible for the abnormalities which appeared in the milk and tissues of certain quarters of some of the animals. Various procedures were followed in order to secure a more complete picture of the flora of the udders than could be obtained by the ordinary ones designed to detect streptococci.

The apparent lack of consistency in the occurrence of any recognized type of bacteria has led to the tentative conclusion that no organism in the cultures was responsible for the changes noted in the secretion and tissues. The pathology of the abnormality, which in previous papers has been called nonspecific mastitis, has already been presented (10), as has its possible relationship to infection of the udder with streptococci (9).

DETAILED FINDINGS

Eleven of the original eighteen cows were observed during three lactation periods. During the first period observations were made each week. In the second and third periods the time between the samplings was somewhat longer on the average. It is impossible to present the detailed data for even a part of the group. In order that a sufficiently complete picture might be presented each lactation period has been divided into 60-day intervals and the average percentage of chlorine for all samples taken in any one interval has been calculated, as have the catalase value and daily production of milk by each quarter. The values for the last sample taken from each quarter in each period are also given in the tables. The use of averages eliminates the effect of transitory deviations from the normal and presents the trend of the continuous changes more clearly.

It is regretted that observations on the productivity of the quarters were not made in the first part of the first lactation period and the last part of the third. The data are, however, sufficiently complete to give a general picture of the conduct of each quarter. Of the

11 animals 4 were considered to be normal throughout the time of observation as regards the condition of their udders, while 7 became abnormal at some time and in some degree during the time of observation. The detailed records of 2 of the 4 normal cows and of 3 of the 7 abnormal cows are presented in the following tables.

NORMAL COWS

The record of cow 1 is given in table 1. It is to be noted that an increase in the percentage of chlorine is evident by the one hundred and eightieth day of the first lactation period; that the increase becomes greater as the period passes; that at the beginning of the second

TABLE 1.—Yield, chlorine content, and catalase value of foremilk, at different stages in a lactation period and in different periods, for each quarter of normal cows 1 and 10

COW 1

Lactation period and interval ¹ No.	Quarter 1			Quarter 2			Quarter 3			Quarter 4		
	Yield per day	Chlorine	Catalase value	Yield per day	Chlorine	Catalase value	Yield per day	Chlorine	Catalase value	Yield per day	Chlorine	Catalase value
First:	<i>Lb.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Lb.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Lb.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Lb.</i>	<i>Pct.</i>	<i>Pct.</i>
1.....		0.110	9		0.105	8		0.107	10		0.113	10
2.....		.103	8		.105	16		.105	10		.097	12
3.....	4.3	.111	18	4.9	.114	18	4.8	.113	8	3.8	.111	7
4.....	3.6	.122	12	4.2	.133	13	4.2	.128	10	3.2	.132	11
5.....	2.8	.138	13	3.4	.137	10	3.5	.145	10	2.4	.146	13
6.....	2.2	.150	13	2.9	.142	10	3.0	.165	42	2.0	.123	22
361 ²	2.2	.177	32	3.2	.161	36	3.5	.216	150	2.5	.203	83
Second:												
1.....	5.5	.109	22	5.4	.124	23	6.1	.123	24	4.4	.123	28
2.....	4.0	.151	22	3.6	.159	21	4.7	.112	26	3.1	.119	28
3.....	2.6	.163	36	3.1	.137	22	3.6	.164	44	2.3	.114	27
4.....	1.7	.171	94	3.1	.173	50	2.3	.221	100	1.5	.176	56
5.....	.9	.224	56	1.8	.200	23	1.1	.252	93	1.2	.205	29
271 ²6	.247	68	1.4	.210	33	1.4	.254	94	1.1	.220	31
Third:												
1.....	8.7	.136	44	10.2	.142	41	7.5	.168	71	7.3	.115	60
2.....	7.3	.160	58	9.0	.150	50	7.1	.159	47	6.4	.151	40
3.....	3.6	.219	110	5.3	.183	60	5.1	.198	70	4.1	.207	65
4.....		.268	159		.229	93		.236	115		.254	132
204 ²255	167		.229	87		.233	119		.259	103

COW 10

First:												
1.....		0.115	5		0.115	4		0.112	5		0.113	5
2.....		.123	9	8.7	.121	4	9.3	.116	5	7.6	.121	8
3.....	6.4	.123	6	7.4	.132	19	7.8	.129	15	6.3	.127	6
4.....	6.1	.150	16	7.2	.161	31	7.1	.154	17	6.3	.151	10
5.....	5.4	.144	12	6.1	.151	26	6.6	.142	8	5.1	.146	8
6.....	3.4	.186	69	4.6	.168	45	4.3	.168	22	3.2	.180	31
359 ²	2.6	.234	80	3.2	.212	104	2.5	.207	39	1.7	.226	36
Second:												
1.....	9.6	.110	18	10.2	.127	41	11.2	.111	19	8.0	.108	13
2.....	8.5	.131	28	8.1	.134	32	10.7	.118	8	9.1	.156	20
3.....	7.8	.144	50	7.3	.150	52	9.6	.134	23	7.5	.131	33
4.....	6.6	.165	48	6.2	.170	44	8.0	.164	33	6.7	.150	21
5.....	4.2	.200	38	4.3	.204	36	4.3	.190	34	4.3	.175	81
6.....	3.8	.227	53	2.5	.182	58	3.9	.230	42	2.8	.214	39
330 ²	4.5	.259	94	2.7	.280	104	3.6	.287	84	2.8	.245	47
Third:												
1.....	9.6	.166	37	11.7	.157	25	13.6	.153	15	9.2	.152	21
2.....	9.5	.188	92	12.3	.163	25	13.7	.146	12	8.7	.147	18
3.....		.190	60		.160	24		.156	17		.163	35
4.....		.187	82		.168	44		.168	29		.158	17
5.....		.197	41		.199	33		.192	32		.171	20
6.....		.202	50		.196	38		.208	42		.187	24
309 ²207	47		.202	33		.205	31		.185	19

¹ Interval is 60 days.

² The day of last sampling. It also gives length of the last interval, e. g., in second period for cow 1, 4 intervals plus 31 equals 271 days.

period it is higher in three quarters than at the beginning of the first; and that the increase is earlier in the second period than in the first and reaches a higher level at the end. The percentage of chlorine is higher at the beginning of the third period than at the first part of the second. A parallel increase during any period, and from period to period, occurs in the case of catalase.

The data in table 2 are the averages of all quarters for chlorine and catalase for the first 60-day interval of each period, and for the fourth interval. The samples from which the data were obtained consisted of the first 30 cc. of milk from each quarter. In previous work both the foremilk and the entire quantity produced by a quarter were examined. The entire milk of a quarter tended to show the same progressive change in chlorine and catalase content as did the foremilk, but to a less extent. This indicates that the change in composition is not caused by increased excretion of fluid from the membranes lining the milk ducts, but is the result of some modification in the synthesis of the milk.

TABLE 2. *Chlorine content and catalase value of foremilk during 2 intervals of 3 lactation periods for normal cows 1 and 10*

Lactation period	Cow 1				Cow 10			
	Interval 1		Interval 4		Interval 1		Interval 5	
	Chlorine	Catalase value	Chlorine	Catalase value	Chlorine	Catalase value	Chlorine	Catalase Value
	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
1	0.109	9	0.129	12	0.114	5	0.146	13
2	.120	24	.185	75	.114	23	.192	22
3	.140	54	.247	125	.157	47	.190	31

It is evident from the values given that cow 1 would have been judged as definitely mastitic during the last of the second period, and during the third after the one hundred and twentieth day, since a composite sample would have given chlorine and catalase values in excess of commonly accepted standards. It is believed that cow 1 may be considered normal, on the basis that all quarters change in much the same manner throughout each period and from period to period.

Little (6) presented data of similar significance. His observations were made on cows yielding milk which failed to show mastitis streptococci, or alteration due to staphylococci or miscellaneous organisms. The data were for groups of cows and showed an increase in chlorine and in leucocytes as the lactation period passed, and from one period to the succeeding. It was also noted by Little that the bacterial content increased in a period, and from period to period, a conclusion which the findings of the present writers tend to confirm. It thus seems that in some cows with apparently normal udders there is a slowly progressive change which is reflected in the composition of the milk, and in a lessened inhibitory power toward bacteria. It may be that herein lies the explanation for the higher incidence of infection of the udder with streptococci in older than in younger animals, rather than in greater opportunity for infection because of longer time. Cow 1 produced 5,193 pounds of milk in 270 days of the

first period and only 3,306 in the same interval of the second. During 205 days of the third period, 5,093 pounds were produced as compared with 2,961 in the same number of days of the second. The cause of the small production in the second period is unknown, and was not evident in the appearance of the animal.

The record of cow 10 is also presented in tables 1 and 2. The changes are similar to those noted in cow 1, but are of less magnitude in any interval. The udder of this animal resisted change to a greater extent than did that of cow 1. This resistance is reflected in the yield of milk, which increased more than 22 percent from the first to the third period. The two remaining normal animals showed changes similar to the two cows just discussed. The quantitative shifts in composition were greater than those in the milk of cow 10 and less than those in that of cow 1. It seems unnecessary to present the detailed data.

ABNORMAL AND INTERMEDIATE ANIMALS

The record of cow 18 is given in table 3. The outstanding points of the record for the first period are the high chlorine and catalase after the three hundredth day, and the apparent normal values previous to this time. The extent of changes was much the same in each quarter. In the first interval of the second period only one quarter, 4, is normal, the other three having an average chlorine content of 0.209 percent as compared with 0.127 percent in the first period. The abnormality increased in intensity during the third period in quarters 1 and 2; quarter 3 changed from abnormal to normal; and quarter 4 from normal to abnormal in the third period. This is one of the few cases of reversion to normal noted in the writers' observation of two groups of cows.

Quarter 3 which changed from abnormal to normal, produced in the second interval of the second period 24.6 percent of the total yield of milk, and in the third period, 33.9 percent. Quarter 4, normal in the second period, and abnormal in the third, produced 25.5 and 21.2 percent in the second interval of these periods, respectively, the changes in the milk were not the same in the different quarters, a condition which may have indicated the presence of some agent not present in cows 1 and 10. The total production for the first 250 days of each period was 7,034, 7,342, and 6,389 pounds, respectively.

The record of cow 8 is also given in table 3. The quarters do not present the same picture in the first period; quarter 3 was abnormal after the first interval, and quarter 1 after the second. The base level of chlorine for the animal was 0.119 percent. In the first interval of the second period, 0.260 percent of chlorine was found, and 0.278 percent in the third period. The values for catalase were 24, 137, and 126. The cow produced in the first 90 days of each of the three periods 4,437, 3,986, and 4,524 pounds of milk, respectively.

The record of cow 7, given in table 3, represents an intermediate between the normal and the distinctly abnormal. The third quarter in the third period produced milk far exceeding the usual standards of chlorine and catalase. The yield of milk decreased from 7,006 pounds in the first 250 days of the first period to 6,584 pounds in the second; and to 5,451 pounds in the third, evidencing a progressive deterioration of the udder which is reflected in the composition of the milk in regard to chlorine and catalase.

TABLE 3.—Yield, chlorine content, and catalase value of foremilk, at different stages in a lactation period and in different periods, for each quarter of abnormal cows 18 and 8, and intermediate cow 7

Lactation period and interval ¹ No.	Quarter 1			Quarter 2			Quarter 3			Quarter 4		
	Yield per day	Chlorine	Catalase value	Yield per day	Chlorine	Catalase value	Yield per day	Chlorine	Catalase value	Yield per day	Chlorine	Catalase value
	Lb.	Pct.	Pct.	Lb.	Pct.	Pct.	Lb.	Pct.	Pct.	Lb.	Pct.	Pct.
First:												
1.....	0.132	11		0.122	11		0.127	6		0.131	11	
2.....	7.8	129	9	9.8	123	6	6.9	137	9	5.8	137	8
3.....	6.5	145	12	7.2	120	3	6.4	141	9	5.5	142	11
4.....	6.8	157	7	7.6	177	24	5.9	105	10	5.1	169	15
5.....	3.9	174	21	5.3	216	64	4.2	187	27	3.5	172	15
6.....	1.1	361	116	1.6	339	123	1.2	343	135	1.1	313	123
319 ²5	378	98	.9	360	110	—	376	133	—	329	138
Second:												
1.....	9.9	200	98	11.2	202	108	10.4	225	116	10.2	125	27
2.....	7.3	207	140	9.1	229	115	8.1	249	123	8.4	126	21
3.....	5.8	207	123	7.5	217	131	6.3	253	129	7.0	136	18
4.....	5.0	240	108	6.5	242	92	5.0	257	74	6.4	157	18
5.....	—	306	190	—	370	176	—	364	190	—	287	122
257 ²	—	398	180	—	396	192	—	400	188	—	371	192
Third:												
1.....	9.5	271	158	7.2	317	165	12.6	177	38	7.7	250	150
2.....	6.6	271	173	6.1	340	208	9.6	184	54	6.0	280	151
3.....	—	242	129	—	275	146	—	175	33	—	272	158
4.....	—	270	126	—	240	94	—	196	62	—	281	106
5.....	—	276	133	—	290	100	—	307	127	—	315	136
283 ²	—	287	139	—	275	116	—	310	139	—	283	119
ABNORMAL COW 8												
First:												
1.....	6.2	0.121	9	9.2	0.129	16	8.7	0.140	51	7.0	0.127	20
2.....	5.4	159	13	7.5	138	6	6.2	232	63	5.7	170	26
3.....	3.3	206	51	6.2	145	10	4.6	279	107	4.6	185	29
4.....	2.1	264	104	4.1	222	44	3.7	258	113	3.2	204	64
5.....	1.0	244	108	2.5	247	94	1.3	260	117	1.5	225	107
268 ²	1.0	253	102	1.9	257	97	.7	265	126	1.5	265	118
Second:												
1.....	6.8	263	88	8.9	289	182	9.1	272	156	7.5	216	121
2.....	5.4	341	137	7.4	287	133	7.6	320	135	6.4	303	131
3.....	1.8	362	188	3.7	320	182	4.1	333	182	3.1	330	180
4.....	—	329	143	—	309	137	—	310	113	—	361	185
194 ²	—	317	172	—	320	139	—	315	144	—	345	191
Third:												
1.....	—	256	100	—	321	163	—	290	150	—	246	91
2.....	—	285	152	—	330	187	—	287	139	—	176	102
3.....	—	300	132	—	297	143	—	264	88	—	261	89
4.....	—	299	133	—	196	120	—	293	124	—	297	126
204 ²	—	287	141	—	282	131	—	276	135	—	286	119
INTERMEDIATE COW 7												
First:												
1.....	0.118	15		0.120	10		0.127	22		0.118	32	
2.....	7.6	110	7	8.3	117	10	7.8	130	14	7.4	107	6
3.....	6.8	114	7	7.4	129	15	6.8	148	23	6.5	117	8
4.....	5.2	140	9	6.0	152	17	5.5	183	30	5.3	135	10
5.....	2.8	152	23	3.0	169	23	3.0	176	28	3.0	165	42
283 ²9	200	60	1.3	214	37	1.2	212	38	1.0	209	51
Second:												
1.....	9.4	.081	11	10.2	.093	19	8.5	.104	30	8.0	.084	10
2.....	8.0	.124	91	9.2	.104	44	7.6	.111	27	7.5	.102	24
3.....	4.8	.188	140	7.3	.118	38	6.4	.151	55	6.4	.140	17
4.....	3.2	.244	144	5.8	.163	46	5.6	.208	73	6.2	.158	33
5.....	.9	.286	—	2.1	.317	—	2.1	.302	—	1.2	—	—
250 ²9	.286	—	2.1	.317	—	2.1	.302	—	1.2	.279	—
Third:												
1.....	8.4	.143	32	9.9	.170	34	7.3	.202	80	5.1	.175	91
2.....	7.2	.138	29	7.3	.170	28	5.5	.203	72	4.7	.170	30
3.....	—	.146	53	—	.209	64	—	.251	125	—	.189	53
4.....	—	.211	93	—	.237	105	—	.244	146	—	.240	84
5.....	—	.276	136	—	.287	126	—	.304	188	—	.280	102
243 ²	—	.276	136	—	.287	126	—	.304	188	—	.280	102

¹ Interval is 60 days.² See footnote 2, table 1.

INCREASES IN CHLORINE

Table 4 shows that the milk produced by the individual quarters of each animal has, in general, a higher content of chlorine in the third than in the first interval of any period. This is the increase usually ascribed to late lactation. The data show that evidences of such changes appear earlier than is commonly supposed.

TABLE 4.—Chlorine content of foremilk from each quarter of five cows at different intervals of the same lactation period and in different periods

Cow No.	Period	First interval				Third interval			
		Quarter 1	Quarter 2	Quarter 3	Quarter 4	Quarter 1	Quarter 2	Quarter 3	Quarter 4
		Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
1.....	1	0.110	0.105	0.107	0.113	0.111	0.114	0.113	0.111
	2	.109	.124	.123	.123	.163	.137	.164	.114
	3	.136	.142	.168	.155	.219	.183	.198	.207
10.....	1	.115	.115	.112	.113	.123	.132	.129	.127
	2	.110	.127	.111	.108	.144	.150	.134	.131
	3	.166	.157	.153	.152	.180	.160	.156	.163
7.....	1	.118	.120	.127	.118	.114	.129	.148	.117
	2	.081	.093	.104	.084	.188	.118	.151	.140
	3	.143	.170	.202	.175	.146	.209	.251	.189
8.....	1	.121	.129	.140	.127	.206	.145	.279	.185
	2	.263	.289	.272	.216	.362	.320	.333	.330
	3	.256	.321	.290	.246	.300	.297	.204	.261
18.....	1	.132	.122	.127	.131	.145	.129	.141	.142
	2	.200	.202	.225	.125	.207	.217	.233	.136
	3	.271	.317	.177	.250	.242	.275	.175	.272

A comparison of the data shows the increase from period to period even in the animals classed as normal in all their quarters. In the case of the second period as related to the first the increase is slight or absent, but generally marked in the third period as compared with the first and second.

Table 5 shows that increases in catalase similar to those in chlorine occur in the milk of individual quarters in any period and from one period to the next. The increase in the cellular content of milk as the lactation period passes was noted by Russell and Hoffman (13) and more recently by Little. Russell and Hoffman also noted the increase in the cell content of the milk as the animal became older. According to prevailing theories chlorine and lactose must change together in order to maintain the milk in osmotic balance with the blood. The question as to which is the cause and which the effect cannot be answered. No matter what the answer, the fundamental process of lactose manufacture is interfered with. It is easy to conceive that the decrease in lactose is the cause of the increase in chlorine in such animals as the writers have considered normal, while in the case of a definite mastitis caused by some foreign element present in only a part of the quarters, the influx of chlorine may cause a disturbance in the physiology of lactose manufacture. Two phenomena are illustrated in the animals discussed herein: (1) An inflammatory process; (2) a physiological one, a part of the reproductive cycle of the animal. The latter seems to be accentuated from period to period. The milk becomes lower in nutritive value, and hence from certain points of view the change may be looked upon as one of deterioration of the udder.

TABLE 5.—*Catalase value of foremilk from each quarter of five cows at different intervals of the same lactation period and in different periods*

Cow No.	Period	First interval				Third interval			
		Quarter 1	Quarter 2	Quarter 3	Quarter 4	Quarter 1	Quarter 2	Quarter 3	Quarter 4
		Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
1	1	9	8	10	10	18	18	8	7
	2	22	23	24	28	36	22	44	27
	3	44	41	71	60	110	60	70	65
10	1	5	4	5	5	6	19	15	6
	2	18	41	19	13	50	52	23	33
	3	37	20	15	21	60	24	17	35
7	1	15	10	22	32	7	15	23	8
	2	11	19	30	10	140	38	55	7
	3	32	34	89	91	53	64	125	53
8	1	9	16	51	20	31	10	107	29
	2	88	182	156	121	188	182	182	190
	3	100	163	150	91	132	143	88	89
18	1	11	11	6	11	12	3	9	11
	2	98	108	116	27	123	131	129	18
	3	158	165	38	150	129	146	33	158

The histological changes found in the tissues of such quarters as 1 and 2 of cow 18 in the second and third periods have been described in a previous paper (10).

MILK PRODUCTION

The influence of any disturbance in the udder on milk production is difficult to determine since so many factors may be concerned. It has been stated earlier that an abnormal quarter seems to produce less than the share of milk it produced when normal. It is possible to calculate from the data presented the part of the total milk produced by each quarter at any interval in any period. The question of compensation cannot be answered in any case, and thus the question of total production in its relation to the condition of the udder, as indicated by changes in the milk, may be the most significant fact. Eleven cows were observed during three periods in such detail as to form a basis for judgment as the condition of the udders. The 11 animals were placed in 3 groups: One containing the normal cows, another the most abnormal, and a third of intermediate condition. The production in the same number of days in the first and second periods is presented in table 6 in percentages of increase or decrease and a similar comparison is made for the second and third periods.

TABLE 6.—*Percentage changes in the production of milk in successive lactation periods by cows classified as normal, slightly abnormal, and very abnormal*

Classification	Number	Increase (+) or decrease (−) in production from—	
		First to second period	Second to third period
Normal cows.....	4	Percent +16	Percent +24
Slightly abnormal cows.....	5	+4	+12
Very abnormal cows.....	2	−3	+2

DISCUSSION

The tissue of the lactating udder of the cow seems very responsive to stimuli, or, in other words, is easily irritated. It is conceivable that a mild irritant acting through long periods of time may cause a noticeable disturbance in function and in structure. The milking machine under certain conditions and on certain cows may exert such a continuously irritating effect. Bacteria of certain types may produce slightly injurious compounds which, acting over considerable periods, may cause a detectable effect, and especially when they grow more freely than in the usual animal. The writers' observations suggest that the bacteria are held in check more completely during the first lactation period than during subsequent ones.

Many agents may be cooperating to produce such changes as are described in this paper, and it may well be that students of mastitis are thinking in too narrow terms, a danger presented by Shope (14, *p.* 932):

Another fact of considerable interest that was not emphasized is that in the case of each one of the seven diseases discussed a simple "one agent" causation was in the beginning considered probable or proved. Some minor discrepancy or fortuitous observation led eventually to the discovery that two agents were involved. This seems to indicate that those who are studying infectious diseases are thinking largely in terms of one instead of two causal agents. They are still under the influence of the spirit of Koch's postulates and find it difficult to abandon even occasionally the concept that for each infectious disease there must be a single specific etiologic agent. If there is anything at all to be learned from present knowledge of the complex infections, it is that an infectious agent must fully explain and account for all of the features of a disease with which it is associated before it is accepted as the sole cause of that disease. Investigators must think more often in terms of two factors if they are to gain full understanding of all the infectious diseases.

The observations presented in this and in previous papers have raised questions rather than answered them. It is evident that dairy cows can be managed intelligently, as regards the avoidance of deterioration of the udder, only with more complete knowledge of the factors involved and of their relations to each other. Munch-Petersen (8, *p.* 238) concludes his recent survey of the literature on bovine mastitis with the statement that much of the work on the subject has been abortive because of lack of fundamental knowledge regarding many aspects of the subject. He emphasizes the necessity of additional research work on a number of points and states that among the conditions which must obtain in order to secure the necessary data—

the herd must be newly established, and be founded by heifers coming to their first lactation, and must be handled for several seasons for the convenience of the research workers and the acquisition of knowledge relating to mastitis, rather than as a commercial concern; and the research workers must have laboratory facilities actually on the premises.

The observations made at this station on two groups of cows emphasize the need which Munch-Petersen presents.

SUMMARY

Eleven Holstein-Fresian cows were observed during the first, second, and third lactation periods as to the chemical composition of the fore-milk and the number and kinds of bacteria therein.

This group was not in contact with other cattle during the period of study. The ration of any member of the group was uniform throughout the period and was the equal, quantitatively and qualitatively, to the rations used on many dairy farms. The cows were milked by a machine, operated by an experienced operator. On 1 day of each week each cow was milked with the machine so arranged as to permit a determination of the yield of each quarter. Samples were taken at weekly intervals throughout most of the period of observation and were examined as to their chlorine content and catalase value and for number and kinds of bacteria. It is considered that the values found in that part of the first lactation period immediately after the colostric stage represent the normal or base level of chlorine and catalase for the individual. On account of the relation existing between chlorides and lactose, the two substances that keep the milk serum in isotonic balance with the blood serum, a marked and persistent increase in the chlorine content of the milk implies some degree of deterioration of the udder, as does an increase in catalase, which is considered to be an indirect measure of the content of the milk in cells or cellular debris.

In the case of each cow the percentage of chlorine and catalase increased as each lactation period passed, and from period to period.

In some cases the increase became evident at about the same time in each quarter and was of much the same magnitude in each. The chlorine content and catalase value then tend to be much the same during the early part of the second period as during the first part of the first lactation period, but reach significantly higher levels during the early part of the third period. The average percentages of chlorine and catalase noted in the middle of the third lactation period were above the values usually considered as normal. The yield of milk showed from period to period the expected or normal increase.

In other cases the normal level of chlorine and catalase of the early part of the first lactation period was exceeded in the early part of the second as well as in the first part of the third period. In such animals the milk produced by the different quarters of the udder did not change at the same time and to the same extent. The deterioration of the udders was then more marked than in the case of the animals classed as normal, as was shown by the fact that the yield of milk did not show the expected increase from the first to the second and from the second to the third periods.

The results of the bacteriological studies, continued throughout the three lactation periods and obtained by various techniques, supplied no explanation for the changes noted. The udder of each animal remained free from streptococci through the three lactation periods. No other organism was found with sufficient consistency and in sufficient number to be apparently responsible for the changes noted in the milk and in the structure of the udder. The observations indicate that the causes of mastitis or of deterioration of the udder may be more complex than is now supposed and indicate the need of a more detailed and more prolonged study of the subject.

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INFLUENCE OF THE PHYSIOLOGICAL AGE OF THE PEA PLANT ON ITS RECOVERY FROM APHID DAMAGE¹

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INTRODUCTION

During 1938, 335 lots of peas comprising 275 varieties varying in earliness of maturity were tested for resistance to the pea aphid (*Illinoia pisi* Kalt.). The aphid population, which had increased rapidly and reached destructive numbers during the test, was suddenly destroyed, apparently by fungi. The extent of recovery of the plants after the disappearance of the aphids appeared to be markedly influenced by the stage of maturity or "physiological age" of the respective varieties at the time of the infestation. The results of an analysis of this relationship is presented.

MATERIALS AND METHODS

The peas were planted May 1 in plots consisting of nine rows 6 feet long and 8 inches apart. Each plot was 2 feet from the adjacent plots in each direction. The seeds were planted by hand, about 1½ inches apart in the row. All plots were infested artificially on May 23 from an aphid stock previously reared in a greenhouse. This infestation, applied 2 weeks before the anticipated peak of spring migration from alfalfa, was believed to be sufficient to seriously injure all except the most resistant varieties. The identification of the resistant varieties, in this way, was anticipated.

RISE AND DECLINE OF THE APHID POPULATION

Cool and rainy weather kept the aphid population low until early June, when conditions changed and the aphid counts showed rapid increases. On June 22, an average of 2,575 aphids was recorded for each 360° sweep with an 11-inch net. This number marked the peak of aphid infestation during the season. Coincident with several warm rains between June 20 and 25, an outbreak of fungous disease occurred among the aphids, and the rapidity and completeness of their disappearance was unusual. Net sweepings on June 30 showed an average of only 39 aphids per sweep. The aphids had reached damaging numbers by June 14 and continued to cause injury until about June 24, making a total damage period of about 10 days. A graph of the 1938 aphid population on peas is shown in figure 1.

NATURE OF INSECT DAMAGE TO PLANTS

The effect of the infestation was fairly uniform throughout the plots. The aphids concentrated upon the apical parts of the plants, injuring the tissue produced just before and during the time of heaviest infes-

¹ Received for publication July 29, 1939. This work was supported in part by a fund from the Associated Seed Growers, Inc., of New Haven, Conn.

tation. These young and succulent vegetative parts became permanently shriveled and malformed. Well-formed pods were stunted and deformed, while very young pods and blossoms were blighted and fell. Several varieties dropped their blossoms even before the attack became severe. Others held their young pods until the pedicels had lengthened, the pods then falling. Still other varieties retained their pods throughout the infestation, the pods becoming twisted and dwarfed and producing at most, one or two peas. Spindly-stemmed varieties such as Alaska and Wisconsin Early Sweet suffered most, the growing tips being killed. Succulent types like Onward and Prince of Wales, although stunted and apparently dead at the apex, recovered.

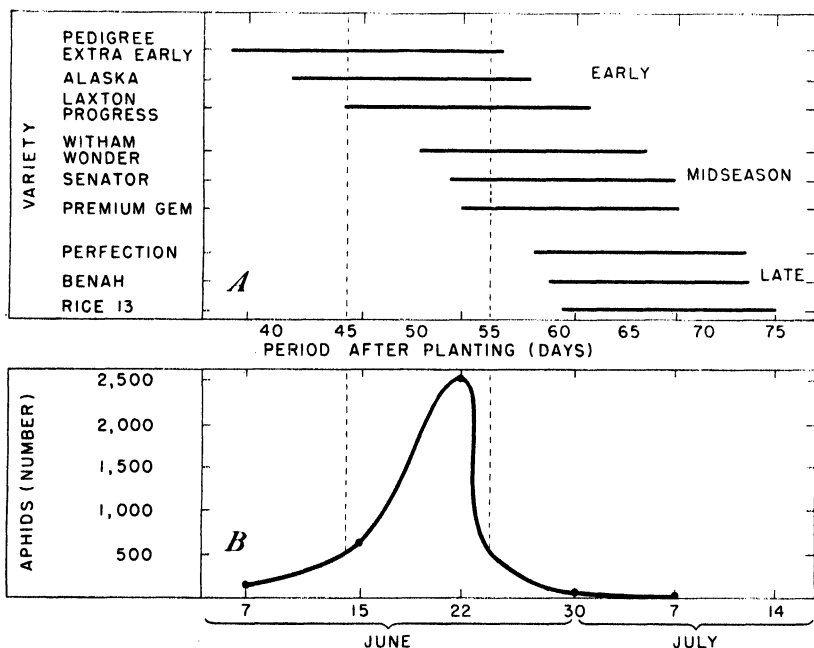


FIGURE 1.—Relation between the period of highest aphid infestation and the stage of development of three maturity groups of canning peas. In A, the length of time elapsing, and the relative period, from full blossom to canning stage of three varieties in each of the three maturity groups is presented in juxtaposition to the aphid numbers given in B. A smooth curve has been drawn through the experimentally determined points. The dates for these points are given below the figure. Days included between vertical broken lines show period of severe damage.

RELATIVE RECOVERY IN EARLY, MIDSEASON, AND LATE VARIETIES

After the decline of the aphid numbers it was noted that recovery occurred in varying degrees on varieties which could be grouped according to their stage of development at the time of highest aphid population. Since nonrecoveries were predominant among the early varieties while recoveries predominated among the late varieties, it was concluded that resistance was not the principal cause of recovery.

A study was made to determine the incidence of recoveries and non-recoveries among varieties of different maturity ranges. The varieties were grouped into four classes according to the number of days required to reach the canning stage from date of planting. Varieties requiring 55 to 65 days were grouped as early, those requiring 65 to 75 days as midseason, both early and late, and those requiring 75 to 85 days as late. A summation of the varieties into these maturity groups and their reaction after injury is presented in table 1.

TABLE 1.—*Nonrecoveries, partial recoveries, and recoveries from aphid infestation among 335 lots of peas comprising 275 varieties maturing at different times*

Blossom date	Maturity range	Lots showing—		
		Nonrecov- ery	Partial recovery	Recovery
Before June 15.....	Early.....	82	12	8
June 15-25.....	Midseason:			
	Early.....	16	14	18
	Late.....	2	6	86
After June 25.....	Late.....	1	3	87
Total.....		101	35	199

DISCUSSION OF PEA PLANT BEHAVIOR

Peas usually develop new nodes at fairly regular intervals, and without great variation in time between varieties. Alaska and Perfection varieties, if planted together, will develop node No. 10, for example, at about the same date. Alaska normally produces its first blossom at node 10, however, and bears its main crop of pods on nodes 10 to 15. Perfection, the later variety, produces its crop on nodes 16 to 22. When the first pods set have matured, growth ceases and the plants die soon after the seed is ripe.

The period of aphid damage lasted from about the forty-fifth to the fifty-fifth day after planting. In terms of vine growth, it occurred while the parts produced from nodes 13, 14, and 15 were tender and succulent. Aphids were attracted in greatest numbers to these parts and the damage was great. Early varieties, blossoming on nodes 9 to 12, depending on the variety, had set from two to six pods before damage was done (fig. 2, A).

In the midseason varieties, normally producing their first blossoms on nodes 13, 14, or 15, the first blossoms were blasted. Only a few varieties which may have been somewhat resistant were able to set one or two pods during the infestation (fig. 2, B and C). Late varieties which blossom after the fifteenth node escaped both blossom and pod injury. The aphids disappeared before the first blossoms were produced (fig. 2, D). The time in days from blossoming to canning for representative varieties of the three general maturity groups is thus correlated with the period of aphid damage in figure 1.

CORRELATION OF PLANT GROWTH STAGE AND RECOVERY

Before the aphid population disappeared, early varieties had produced some well-developed seed. These plants were physiologically nearing maturity although no older in point of time from planting or



FIGURE 2.—Line drawings made over photographs taken July 7, 1938. The effect of the severe aphid damage upon the various maturity groups is shown between the heavy horizontal lines. A, Laxton Progress, early; B, Witham Wonder, early midseason; C, Premium Gem, late midseason; D, Benah, late. Extent of growth above upper line indicates amount of recovery.

node development than other varieties. Canning peas do not ordinarily regenerate after they have reached maturity. After the aphids had been destroyed there was little or no new growth in this group of peas.

Midseason varieties had reached from early to full bloom during the period of damaging aphid numbers. For the most part, all blossoms and young pods present were so injured at this time that they fell from the vines. The amount of growth produced after the period of damage was apparently limited by the age of the first surviving pods produced. If a variety retained one or two well-developed, even if malformed, pods during the infestation, there was little regeneration. If a variety had set no pods prior to the infestation, new growth continued until the first-formed postinfestation pods became mature. Several varieties put out adventitious shoots from nodes immediately below the injured area (fig. 2, *C*). Those varieties that were able to produce and hold some pods during the infestation, actually produced a smaller yield than those that were unable to produce pods during the damage period. Those midseason varieties that lost all of their reproductive parts as a result of the aphid attack regenerated when the aphids were destroyed. Blossoms were produced at later nodes and an apparently normal set of pods followed.

Late varieties, although retarded by two or three shortened and weakened internodes, soon outgrew the damage. Few blossoms had been produced by the late varieties at the period of damage, and those that had been produced were blasted and fell from the plant. After the aphids had disappeared, the relatively uninjured growing tips quickly resumed growth. When the first blossom nodes were reached, blossoming began and the plants produced an apparently normal crop of peas.

CONCLUSIONS

From these studies it has been concluded that the instance described above is a clear case of escape and that the ability of varieties in certain maturity groups to produce a satisfactory crop of peas is not evidence of resistance. Escape, such as described above, may not be depended upon to protect crops from insect injury and is therefore of little value to the plant breeder or entomologist who is trying to produce resistant varieties. The phenomena described are of significance in two outstanding respects: (1) They indicate that comparative yield alone may not be depended upon as a measure of the ability of the plant to resist insect attack, and (2) that the physiological age of the plants, not resistance, appears to determine the comparative ability of the various maturity groups to recover after an aphid attack has stopped short of actual destruction of some of the varieties.

MINIMUM LETHAL DOSE OF SELENIUM, AS SODIUM SELENITE, FOR HORSES, MULES, CATTLE, AND SWINE¹

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INTRODUCTION

Following the discovery of selenium in numerous plants grown in certain areas of the north-central Great Plains and its identification as the cause of so-called alkali disease of livestock in those areas, a number of experiments have been undertaken to test its toxicity. Most of this work was designed to study the effect of continued feeding of small doses of selenium in various forms to small experimental animals. The disease produced in this way was of the subacute or chronic type. In a few studies, however, large single doses of selenium have been administered, resulting in the acute type of poisoning. It appears that only small laboratory animals have previously been used for this work. Franke and Moxon,³ using rats, found that the minimum fatal dose (smallest dose that killed 75 percent or more of the animals in 48 hours) of selenium, in the form of sodium selenite, injected intraperitoneally was 1.5 mg. per pound of body weight. Of sodium selenate, the minimum fatal dose was found to be 2.5 mg. per pound of body weight. Smith, Stohlman, and Lillie,⁴ using rabbits, found that the fatal dose of selenium, in the form of sodium selenite or selenate, when administered orally was 1.5 to 2.0 mg. per pound of body weight. No reference has been found of any such experimentation with the larger domestic animals.

In order to obtain information on this point, experiments were carried on in 1934 and 1938 at the Animal Disease Station, Beltsville, Md., to determine the minimum lethal dose of selenium for horses, mules, cattle, and swine. This work also afforded an opportunity to observe any symptoms and gross pathological changes which might result from acute selenium poisoning and how the element was distributed in the body tissues. The results of the experiments are reported in this paper.

EXPERIMENTAL PROCEDURE

The number and weights of the different species of animals used in the experiments, the dosages given, and the methods of administration appear in table 1. The animals were normal as far as could be ascertained by clinical examination. Some of the horses and mules, however, had been included in infectious-disease experiments before being used in this work. Horse 775 had been given 5 cc. of a 24-hour culture of *Pasteurella bubaliseptica* a few weeks before it received sele-

¹ Received for publication October 30, 1939.

² The writers are indebted to H. C. Dudley, formerly of the Bureau of Chemistry and Soils, for chemical analyses, for selenium, of the tissues, milk, and urine of several experimental animals. Acknowledgment is also made to G. T. Creech of the Bureau of Animal Industry for histopathological examinations.

³ FRANKE, K. W., and MOXON, A. L. A COMPARISON OF THE MINIMUM FATAL DOSE OF SELENIUM, TELLURIUM, ARSENIC, AND VANADIUM. *Jour. Pharmacol. and Expt. Ther.* 58: 454-459. 1936.

⁴ SMITH, M. I., STOHLMAN, E. F., and LILLIE, R. D. THE TOXICITY AND PATHOLOGY OF SELENIUM. *Jour. Pharmacol. and Expt. Ther.* 60: 449-470. 1937.

nium. Horses 875 and 1003 and mules 991 and 1033 had been exposed to infectious anemia virus. Horse 1009 had been used in several infectious-disease experiments, whereas no previous experimentation had been done on horse 764 or on mule 1035. Although no records were available concerning the ages of the horses and mules, it was evident that they were 8 years of age or more.

TABLE 1.—Summary of experiments to determine the toxicity of single doses of selenium in the form of sodium selenite administered orally to horses, mules, cattle, and swine

Species and No.	Live weight	Dosage of		Method of administration	Selenium as sodium selenite per pound of body weight	Result
		Sodium selenite	Water			
	Pounds	Grams	Cubic centimeters		Milligrams	
Horse:						
775	1, 185	14. 22	100	Drench	5. 50	Death in 24 hours.
764	970	9. 70	500	Stomach tube	4. 60	Death in less than 24 hours.
875	1, 435	11. 48	500	do	3. 65	Do.
1009	1, 450	6. 34	500	do	2. 00	Death in 26 hours.
Mule:						
1033	1, 227	4. 02	500	do	1. 50	Death in 36 hours.
1035	925	3. 03	500	do	1. 46	Death in about 36 hours.
991	850	2. 79	500	do	1. 50	Very sick.
Horse 1003	1, 110	2. 98	500	do	1. 23	Sick.
Calf 1	66	1. 32	50	Drench	9. 15	Death in 6 hours.
Cow:						
1729	1, 050	14. 71	85	do	6. 40	Death in about 48 hours.
1912	1, 360	16. 32	95	do	5. 10	Death in about 30 hours.
1914	705	7. 05	75	do	4. 57	Very sick.
1839	710	5. 00	50	do	3. 20	No effect.
Pig:						
4282	53	1. 20		Feed	10. 30	Death in 72 hours.
4754	130	2. 28	35	Drench	7. 90	Paralyzed; killed after 18 days.
4737	100	1. 31	35	do	6. 00	Sick.
4730	120	1. 57	20	do	6. 00	Do.
4683	120	1. 05	20	do	4. 00	Do.
4703	120	. 52	15	do	2. 00	Slightly sick.
4746	150	. 33	15	do	1. 00	No effect.

Two of the cows, Nos. 1914 and 1839, were in the first lactation period and were between 3 and 4 years old, whereas the other two were several years older. The calf was 5 days old. The pigs ranged in age from 4 to 6 months. None of these animals had been exposed directly to any known sources of infectious disease.

Selenium was given orally to each animal, in the form of sodium selenite, in the feed, as a drench, or by stomach tube. All the dosages were calculated on the basis of body weight. Since no information was available on what constituted a toxic or lethal dose for large animals, a fairly large quantity was given in the beginning and was reduced progressively in subsequent tests on other animals until symptoms of acute poisoning were obtained. The animals of each species (horses and mules being grouped together) are listed in table 1 according to size of the dose, with one exception.

EXPERIMENTAL RESULTS

HORSE 775

Horse 775, which was dosed in the afternoon, was depressed, weak, and trembling on the following morning, and its breath had a distinct odor resembling that of garlic. Death occurred in the afternoon of the same day.

On post mortem few lesions were found. The lungs were expanded and marked with the outline of the ribs, and although the interlobular

connective tissue was easily observed no emphysema was present. The heart contained black, coagulated blood. Black hemorrhages about 1 cm. in diameter were found along the coronary groove and in the ventricles. The intestinal tract was reddened and filled with bloody fluid.

HORSE 764

Horse 764 was treated about 1:30 p. m., at which time it appeared normal in all respects and its temperature was 99.2° F. About 10 a. m. on the following day the temperature had risen to 102°. At this time there were slight spasms of the neck and face muscles and grinding of the teeth. The eyes were dilated and staring. There was profuse sweating with distension of the cutaneous blood vessels, although the pulse was very weak. The nostrils were expanded and the respirations were labored, with accessory intercostal breathing. The animal died about 1 hour later.

On post mortem a few small hemorrhages were found on the visceral pleura and the surface of the lungs. There were a few petechiae on the pericardium, and in the heart itself multiple hemorrhages occurred under the endocardium, which extended slightly into the myocardium. The liver showed slight diffuse fatty degeneration. A few hemorrhages, as well as severe gastritis, were found in the stomach. There was marked enteritis. A few subcapsular hemorrhages were noted on the spleen. No marked changes were present in the kidneys, but the bladder showed a diffuse cystitis.

Chemical analysis of the tissue of this animal was made for selenium. The results appear in table 2.

TABLE 2.—Selenium content of body parts and fluids of animals that received single doses of selenium

[Results given in parts of selenium per million, based on weight of samples of body parts and fluids taken on post mortem]

Body part or fluid	Selenium content ¹ of indicated body part or fluid of—										
	Horse No.			Mule No.		Calf No.	Pig No.				
	764	875	1009	1033	1035	1	4282	4703	4730	4737	4754
	Parts per million	Parts per million	Parts per million	Parts per million	Parts per million	Parts per million	Parts per million	Parts per million	Parts per million	Parts per million	Parts per million
Bile.....						5.0					
Blood.....	2.0	2.5		0.8	2.0						2.5
Blood and lymph.....						27.0					
Bone (left humerus).....							T.				
Bone (rib).....							T.				
Bone marrow (right femur).....							5.0				
Brain.....	.2	.4									.3
Colon and contents.....							50.0				
Feces.....	8.0				10.0						
Gall bladder.....											2.0
Heart.....	.4	.4	0.5	.3	1.0	16.0	.05	0.05	0.1	0.5	.1
Hide and hair.....							.0				
Hoof.....	.2	.2									2.0
Intestines (small) and contents.....						14.0					
Intestines (small), tissue.....							5.0				
Kidney.....	10.0	8.0	10.9	2.0	6.0	18.0	.2	.5	.2	.3	
Liver.....	5.0	8.0	2.0	1.5	2.0	25.0	2.5	.1	.2	.2	1.0
Lung.....	1.5	1.5	.7	.5	1.0	8.0	1.0	.05	.5	.2	.2
Muscle, leg.....	.3	.5					T.				.2
Muscle, rib.....							T.				
Spinal cord.....											.1
Spleen.....	.4	.5	.5	.4	1.0	20.0		.05	.05	.1	.2
Stomach tissue.....	20.0	62.0									.5
Urine.....			1.4	20.0	4.0						4.0

¹ T.—trace.

HORSE 875

Horse 875, which was dosed at the same time as horse 764 (about 1:30 p. m.), was found dead at 8 o'clock the following morning.

On post mortem, the carcass was found to be very fat and there were multiple subcutaneous hemorrhages. In the lungs there were small hemorrhages of the visceral pleura. Numerous petechiae were found on the pericardium, and there were subendocardial hemorrhages in the ventricles. In the stomach there was marked hemorrhagic gastritis. The liver was marbled with fatty degeneration. The spleen contained a few subcapsular hemorrhages of varying size. The kidneys appeared more or less normal, but an ulcerative cystitis was present in the bladder.

Chemical analysis of the tissue of this animal also was made for selenium. The results are given in table 2.

HORSE 1009

Horse 1009 was treated at 11:30 a. m. At 4 p. m. of the same day, its temperature was 100.6° F., and the animal seemed normal. At 9 a. m. the following day, the temperature was 101.0°. The animal was breathing very hard, and the pulse was rapid. An hour and a half later the horse was down, the temperature was 103.6°, and tonic spasms were noted. Death occurred at 1:10 p. m., or about 26 hours after the selenium had been given.

On post mortem, the blood was found to be almost black in color and had not coagulated 1½ hours after death. No lesions were observed in the lungs or in the liver. There were ecchymotic hemorrhages along the coronary groove of the heart and subendocardial hemorrhages in the ventricles. The kidneys were friable, and there were hemorrhages in the cortex.

Chemical analysis of the tissue of this animal was made for selenium. The results are given in table 2.

MULE 1033

Mule 1033 was treated about 1 p. m. At 4 p. m. of the same day, the temperature was 102.2° F. The next morning, the temperature was 101.0°, at which time the mule was breathing hard, with nostrils distended. The pulse was very weak. Feed and water were refused. A blood sample collected at this time was black but was not hemolyzed. In the afternoon the temperature was 100.2°. The animal was found dead on the morning of the second day.

On post mortem the lungs appeared more or less normal, except for a few small black hemorrhages under the visceral pleura. There were also many black ecchymotic hemorrhages in the mediastinum at the bifurcation of the bronchi and under the parietal pleura in the intercostal spaces. The heart contained numerous small black hemorrhages along all the coronary vessels as well as on the auricles. In the ventricles a few subendocardial hemorrhages were found. The blood was black and did not coagulate readily. There was some fatty degeneration of the liver. In the spleen, a few subcapsular hemorrhages were found. Areas of acute enteritis appeared along the small intestines, and the blood vessels were distended with black blood. There was an acute nephritis as well as an acute catarrhal cystitis. The urine was coffee colored and contained numerous gelatinous clots.

Chemical analysis of the tissue of this animal was made for selenium. The results are given in table 2.

MULE 1035

Mule 1035 was treated about 11:15 a. m. At 4 p. m. of the same day the animal's temperature was 101.5° F., pulse 76, and the respirations were fast. It had no appetite and appeared sluggish. On the following day, the temperature was 101.6°, the pulse was weak, and the respirations were fast and labored. The animal had not eaten or drunk since the previous day. At 4 p. m. the pulse was 90, temperature was 101.5°, and the respirations were fast and very labored. It was found dead on the morning of the second day.

On post mortem there were large subcutaneous hemorrhages over the ribs. The lungs were congested. Numerous small hemorrhages were present on the fat of the heart, and very large hemorrhages were found along the coronary groove and under the endocardium of the left ventricle. The blood was black but had not coagulated. There was a fibrinous peritonitis on the lower part of the diaphragm and subperitoneal hemorrhages under the processes of the lumbar vertebrae. Numerous fibrinous tags were present on the surface of the liver, which was marbled with large areas of fatty degeneration. Very few petechiae were observed on the spleen. There were hemorrhagic enteritis and large black hemorrhages in the folds of the mesentery. The kidneys were soft and friable.

Chemical analysis of the tissue of this animal was made for selenium. The results are given in table 2.

MULE 991

Mule 991 was treated at 1 p. m. The next morning the temperature was 99.4° F., and the pulse was weak. The respirations were labored and fast, and the nostrils were distended. It did not eat and drank little water. A blood sample drawn at this time was black but not hemolyzed. In the afternoon the temperature was 102.2°, but the general condition remained unchanged. The animal drank a considerable quantity of water on the second morning but did not eat. The temperature was 100°. The breathing, however, seemed normal although the pulse remained weak. From this time on the mule improved, and 7 days after the selenium was given it was eating and drinking and appeared entirely normal.

Chemical analysis of the blood sample showed a selenium content of 1 p. p. m.

HORSE 1003

Horse 1003 was treated at 12:45 p. m. The animal was not eating or drinking on the following day, and there was some diarrhea. It was breathing hard. A blood sample collected on the morning of the second day was very black. At this time, the horse was eating hay and drinking water again and the respiration had returned to normal. There was no change in the temperature at any time, and the horse remained normal for the remainder of the period of observation.

Chemical analysis of the blood sample showed a selenium content of 0.5 p. p. m.

CALF 1

Calf 1 was treated about 9:30 a. m. and died about 3:30 p. m. the same day. At this time there was a discharge from the mouth and a profuse diarrhea. The animal was not autopsied. Chemical analysis of the tissue of this animal was made for selenium. The results are given in table 2.

COW 1729

Cow 1729 was lactating and not pregnant. It was treated about 9:30 a. m., and in the afternoon of the same day was salivating profusely and appeared very dull. It did not eat or drink. The condition was about the same on the following morning, except that there was no salivation. The animal was down in the afternoon, the breathing was labored, the eyes were dull, and the extremities were cold. The breath had a marked garlic odor, and the feces a peculiar fetid odor. The animal was found dead on the second morning.

On post mortem the lymph glands throughout the body were hemorrhagic. Extensive pleural adhesions were found in the thoracic cavity, but the lungs appeared relatively normal. In the heart there were numerous subepicardial hemorrhages which were particularly marked on the left ventricle. Three to four liters of straw-colored fluid was present in the peritoneal cavity. There were numerous hemorrhages about 1 cm. in diameter throughout the liver, in one lobe of which was a circumscribed abscess, about the size of a baseball, containing white fluid pus. A few subcapsular hemorrhages were found in the spleen. Both kidneys showed an acute nephritis with a few small hemorrhages under the capsules, but the urine appeared normal.

A milk sample taken on the first day after ingestion of the sodium selenite contained 0.02 p. p. m. of selenium. On the second day at the time of death the milk contained 0.04 p. p. m. and the urine 5 p. p. m. of selenium.

COW 1912

Cow 1912 was dry and carrying an 8-month-old fetus. It was treated in the morning, and death occurred late in the afternoon of the following day. On post mortem few lesions were found.

COW 1914

Cow 1914 was lactating and was rather thin at the time it was treated. The next day the animal took little feed or water, and milk production was greatly reduced. On the second day the cow was down practically all the time and had stopped giving milk. It did not eat or drink during this time. Two days later, however, it was eating hay and drinking again and also giving a small quantity of milk. From this time until the cow was autopsied 5 days later it ate only hay, drank little water, and seemed to lose slightly in weight. Milk production did not return to the pretreatment level. Post-mortem findings were entirely negative.

COW 1839

Cow 1839 was lactating and in good condition when treated. The animal was observed for the next 20 days, and no clinical symptoms of selenium poisoning were shown at any time. There was no evident

impairment of the appetite for either grain or hay, the usual quantity of water was taken, and milk production remained the same as before treatment. However, the feces had a peculiar fetid odor on the second day after the selenium was given, and on the following day a somewhat similar odor was noted in the milk at the time it was drawn. On post mortem, 20 days later, the animal appeared entirely normal.

On the fifth and sixth days after ingestion of the selenium the milk was found to contain 2 and 3 p. p. m., respectively. The selenium content of the urine on the sixth day was 3 p. p. m.

FIG 4282

Pig 4282 was given dry sodium selenite mixed with a little moistened mill feed, and the mixture was eaten readily. Few clinical symptoms of selenium poisoning were noted during the next 2 days, but the animal was found dead on the morning of the third day.

On post mortem the carcass was in good condition, the hair was firm, but the skin was slightly reddened. The cutaneous blood vessels were filled with dark, coagulated blood. The pleural cavity contained about 150 cc. of clear reddish-yellow fluid. The trachea and bronchi were filled with foam, and some pneumonic changes were found in the lungs. The heart contained a large quantity of black blood, and there were a few hemorrhages along the coronary vessels. About 200 cc. of yellowish fluid was present in the stomach, and the mucosa of the fundic portion was slightly reddened. The intestines appeared practically normal, but the mesentery contained numerous large dark hemorrhagic areas and the mesenteric lymph glands were black.

On histological examination there was extensive congestion in the capillary blood vessels and thrombi in a number of the larger vessels of the liver. Most of the liver cells showed extreme cloudiness with fatty changes, together with cell vacuolation, and many of these cells also contained deposits of bile pigment. Few normal liver cells were observed, but there was no perceptible increase in the interstitial structure of the organ.

In the kidney there was marked capillary engorgement with slight hemorrhages and also thrombi in several of the larger vessels. Changes in the tubular epithelium varied from cloudiness and loss of cell nuclei to complete destruction of the cells. Groups of tubules were seen in various areas which exhibited more advanced degenerative changes than in other parts of the section. Fatty changes with cell vacuolation and exfoliation of the epithelium in many tubules were particularly noticeable in these areas. Varying amounts of pigment were observed in this section.

There was blood engorgement, particularly in the larger vessels of the spleen, and a slight edema. The interstitial tissue appeared to be somewhat increased.

The changes in the lungs were typical of catarrhal pneumonia, such as thickening of the alveolar walls, cellular infiltration, and sloughing of the bronchial epithelium.

Practically all the vessels of the heart muscle were engorged with blood. The muscle fibers showed cloudiness with beginning fatty changes. Rather heavy deposits of greenish-yellow pigment (bile) were observed, particularly along the course of the blood vessels.

Chemical analysis of the tissue of this animal was made for selenium. The results are given in table 2.

FIG 4754

Pig 4754 was treated about 9:25 a. m. Within the next 5 hours, the pig had vomited four times. On the following day the animal was down, shivering, and had a profuse diarrhea. Post mortem was made 18 days later. During that period the pig was unable to move its legs, although it appeared fully conscious and was able to eat when grain was offered.

On post mortem relatively few lesions of any consequence were found. There was some fatty degeneration of the liver and a few hemorrhages in the cortex of the kidneys. Chemical analysis of tissue for selenium is given in table 2.

FIG 4737

Pig 4737 was treated at the same time as pig 4754. On the following day the animal was extremely sick, although it was not observed to vomit at any time. This pig lay down most of the time, ate little, shivered, squealed, and ground its teeth a great deal. On the fourth day the animal was able to move about and appeared somewhat better, and by the fifth day it seemed to have returned practically to normal and was eating again.

On post mortem 2½ months later no lesions were found. Chemical analysis of the tissue of this animal was made for selenium. The results are given in table 2.

FIG 4730

Pig 4730 was treated in the morning. Within the next 24 hours it vomited greenish material several times. At the same time there was considerable depression, no appetite, and disinclination to move about. From this time on the animal recovered gradually until 1 week later it appeared to be entirely normal.

On post mortem, 2½ months later, the only lesions noted were subendocardial petechiae in the left ventricle. Chemical analysis of the tissue of this animal was made for selenium. The results are given in table 2.

FIG 4683

Pig 4683 was treated in the morning. Although this animal did not vomit, on the following day it showed symptoms of selenium poisoning resembling those of the other pigs, but not so pronounced. Two days later it began to eat and within 5 days returned to normal.

FIG 4703

Pig 4703 was treated in the morning. The animal was slightly sick for the next 24 hours, as evidenced by lack of appetite and general sluggishness. The following day the animal appeared to have recovered fully.

On post mortem, 2½ months later, a slight pericarditis constituted the only gross lesion found. Chemical analysis of the tissue of this animal was made for selenium. The results are given in table 2.

FIG 4746

Fig 4746 never showed any clinical symptoms of selenium poisoning.

DISCUSSION

From the results shown in table 1, it appears that the minimum lethal dose of selenium, when administered by mouth in the form of sodium selenite, has been established within reasonably close limits for horses, mules, cattle, and swine. In addition, it has been demonstrated that there is a distinct difference in the ability of the several species of animals to tolerate various quantities of selenium when given orally in single doses. Enough animals were not included in this work to determine whether there is any difference in susceptibility among individuals within a species.

The minimum lethal dose of selenium for horses and mules was determined with somewhat more exactness than that for the other two species. The first three horses to be treated received 3.6 mg. or more of selenium per pound of body weight. The animals survived for 24 hours or less. When the dose was reduced to about 1.5 mg., one mule out of three survived. The two mules that died after receiving about 1.5 mg. per pound of body weight were very fat, whereas the one that survived was thin as a result of having had infectious anemia (swamp fever). However, typical symptoms of acute selenium poisoning were induced by this dose, and a period of about a week elapsed before the mule was eating and drinking normally again. The minimum lethal dose of selenium for horses and mules was therefore about 1.5 mg. per pound of body weight.

It seemed evident from the results obtained that animals in good condition might be more susceptible to the action of selenium than comparatively thin animals. Therefore, in calculating a dose of selenium for an animal with excess weight, if a dose which merely produces symptoms of selenium poisoning in a thin animal of the same weight is reduced in proportion to the excess weight of the former animal, the same results should be obtained in this animal as in the latter one. To test this assumption a fifth horse (No. 1003) was treated. The animal was very fat, its weight being estimated as 200 pounds in excess of normal for a horse of its size and conformation. The quantity of selenium administered was, therefore, reduced by 18 percent. Typical symptoms of selenium poisoning were produced, from which the animal apparently recovered in about 3 days.

The symptoms of acute selenium poisoning in horses are not particularly characteristic. There is first a lack of appetite and refusal to drink water, and the animal stands quietly in the stall. The pulse is slightly accelerated and the respirations are fast. The temperature may be normal or slightly higher. A few hours before death, the animal stands as though fixed to the floor, eyes staring, nostrils dilated, and the breathing is convulsive, fast, and very labored. The temperature has a tendency to rise, and the pulse is fast and weak or imperceptible in some animals. A blood sample taken at this time is almost black in color, but there is no hemolysis.

That death is due to asphyxiation has been mentioned previously by Beath and coworkers.⁵ This explanation seems entirely probable,

⁵ BEATH, O. A., EPPSON, H. F., and GILBERT, C. S. SELENIUM AND OTHER TOXIC MINERALS IN SOILS AND VEGETATION. Wyo. Agr. Expt. Sta. Bul. 206, 56 pp., illus. 1935.

both from the symptoms presented in acute cases of poisoning and from the analysis of fractions of whole horse blood by Dudley.⁶ In his work it was shown that a "protein-like selenium complex" was present in the erythrocytes and none in the serum, plasma, and fibrin. As a consequence of the formation of this new compound in the red cells and in the presence of sufficient selenium, it is possible that the respiratory capacity of the blood is reduced to a point where death follows. A possible explanation for the difference in susceptibility of the three mules which received 1.5 mg. of selenium per pound of body weight is as follows: A fat animal that is given the same quantity of selenium per pound of body weight as a thin one receives a larger dose in relation to its total blood volume because of the avascularity of fat tissue and has a correspondingly poorer chance for survival. There is a possibility, then, that if the dose of selenium could be calculated in terms of blood volume rather than of body weight, the variation in susceptibility among individuals of the same species would not be so pronounced. Furthermore, oxygen-capacity determinations of the blood would perhaps give even more information on this point because of the apparent affinity of selenium for hemoglobin.

The analysis of tissue from the horses and mules that died of toxic doses of sodium selenite brings out some noteworthy points. The selenium content of the stomach, kidneys, and liver of the animals was high as compared with that of the other tissues examined. The high selenium content of the urine and feces of these fatal cases and of chronic cases⁷ shows that large quantities of selenium are eliminated through the kidneys as well as through the intestines. The concentration of selenium in the blood of these animals at death was not so great as in the chronic cases over a period of several months.

In cattle the minimum lethal dose of selenium was found to be between 4.5 and 5 mg. per pound of body weight. This was about three times the quantity required to kill horses. The symptoms of acute poisoning in cattle are similar to those observed in horses, although not so marked.

The analysis of tissue from the calf, which died 6 hours after the administration of 9.15 mg. per pound of body weight, showed a relatively high selenium content throughout the animal.

Swine proved to be even more resistant to the action of single doses of selenium than cattle. One animal (No. 4282), which received 10.3 mg. per pound of body weight, died in 72 hours, and a second pig (No. 4754) was paralyzed, but did not die, after receiving 7.9 mg. Since it had vomited at least four times within 5 hours after dosing, it probably did not retain all the selenium given. Had it received the full effect of the entire dose, it is probable that it would not have survived. Of the two pigs given 6 mg. per pound of body weight, No. 4730 vomited shortly after it was drenched, but there were no indications that No. 4737 had done so. Judging from these results, the minimum lethal dose of selenium for swine is between 6 and 8 mg. per pound of body weight.

⁶ DUDLEY, H. C. TOXICOLOGY OF SELENIUM. I. A STUDY OF THE DISTRIBUTION OF SELENIUM IN ACUTE AND CHRONIC CASES OF SELENIUM POISONING. *Amer. Jour. Hyg.* 23: 169-180. 1936.

⁷ Unpublished data.

The symptoms of acute poisoning in pigs consist largely in lack of appetite, sluggishness, and general depression. Diarrhea was observed in one pig, and grinding of the teeth in another.

On post mortem, in cases of acute poisoning, certain common gross changes were found in all four species of animals. Among these were black-colored blood which did not coagulate readily, hemorrhages in the heart (along the coronary vessels and under the endocardium, particularly in the left ventricle), and small subcapsular hemorrhages on the spleen. Less commonly, large hemorrhages were found subcutaneously, in the mediastinum and in the folds of the mesentery. In a few cases there were marked fatty degeneration of the liver and petechiae in the cortex of the kidney.

The analysis of the tissue from pig 4282 shows that the selenium did not get into the muscles, hide, hair, and bones except in very minute quantities during the 3-day interval between ingestion and death. The blood and urine of pig 4754 contained 2.5 and 4 p. p. m., respectively, 18 days after ingestion of sodium selenite equivalent to 7.9 mg. of selenium per pound of body weight. The elimination was well advanced as the selenium content of the body tissues was low. Examination of pigs 2.5 months after ingestion of relatively large doses of sodium selenite showed the nearly complete elimination of selenium.

SUMMARY

Selenium in the form of sodium selenite was given in large single doses to horses, mules, cattle, and swine to determine the minimum lethal dose of the element for these species. It was administered orally in the feed, as a drench, or by stomach tube, the dose being calculated as milligrams of selenium per pound of body weight. Large doses were given at the beginning of each test and the quantity was reduced for other animals until the minimum lethal dose was reached. The experiments were carried on in 1934 and 1938 at the Animal Disease Station, Beltsville, Md.

Five horses and three mules were used. Although all but two had been used previously in infectious-disease experiments, they appeared healthy on clinical examination. The minimum lethal dose of selenium was found to be about 1.5 mg. per pound of body weight.

In the experiments with cattle, one calf and four cows were included. The calf was 5 days old. Two of the cows were between 3 and 4 years of age and the other two were several years older. All these animals appeared healthy clinically and were not known to have been exposed to any infectious disease. The minimum lethal dose for this species was found to be between 4.5 and 5 mg. per pound of body weight.

In the experiments with swine, seven apparently healthy pigs, varying in age from 4 to 6 months, were used. For these animals the minimum lethal dose of selenium was found to be between 6 and 8 mg. per pound of body weight.

HYBRIDIZATION OF AMERICAN 26-CHROMOSOME AND ASIATIC 13-CHROMOSOME SPECIES OF GOSSYPIMUM¹

By J. O. BEASLEY

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INTRODUCTION

Within the last few years a number of workers have attempted to cross cultivated Asiatic 13-chromosome and American 26-chromosome species of cotton (*Gossypium*). Zaitzev (18)² secured the cross with the 13-chromosome type as female, while Nakatomi (11) produced the cross by using the 26-chromosome type as female. Harland (5), from several thousand reciprocal crosses, secured 2 hybrids with the 26-chromosome type as female. Feng (4) reported having seen 2 natural hybrids between American and Asiatic cottons. He made 1,017 crosses with the Asiatic type as female and secured 1 hybrid. The reciprocal cross gave 5 hybrids from 691 crossed flowers. Feng found that some hybrid seeds contained small embryos and others contained none. When American types were used as female, the capsules dropped within 2 weeks, but when Asiatic species were used as female the capsules reached nearly normal size. Feng determined that pollen tubes reached the base of the styles, and he suggested that the gametes were incompatible. Webber (16) made 125 pollinations between cultivated American and Asiatic cottons without securing any crosses. Doak (3) attempted to secure crosses between Asiatic and American cottons by pollinating some of the stigmas of American cotton with pollen from an American plant and then pollinating the remaining stigmas of the same flowers with pollen of an Asiatic type. He reported that pollen tubes of the Asiatic species entered the embryo sacs, but no seeds developed.

From a review of work on hybridizing American 26-chromosome and Asiatic 13-chromosome species, it is evident that the few crosses secured resulted from using ordinary methods of hybridization and making hundreds of crosses. It was, therefore, desirable to determine the causes of the high degree of abortion noted in the crosses and to find a way of producing the hybrids at will. It seemed necessary to study pollen germination, pollen-tube growth, fertilization, and the embryology of the hybrids.

RESULTS OF CROSS-POLLINATION

TECHNIQUE

A modification of the technique described by Sears (12) was used to study pollen germination and pollen-tube growth. Styles were cut from ovaries 3 to 6 hours after pollination; the epidermis of the fused styles was cut, and the styles were pulled apart; they were then

¹ Received for publication September 25, 1939. Cooperative investigations by the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the North Carolina Agricultural Experiment Station. Part of a thesis problem carried out at Harvard University under the direction of the late Prof. E. M. East.

² Italic numbers in parentheses refer to Literature Cited, p. 180.

dropped in boiling 4-percent sodium sulfite solution and boiled 3 to 4 minutes; then washed a few minutes before they were placed in a solution of chloral hydrate (saturated solution diluted with an equal volume of water), to which a few drops of basic fuchsin was added as a stain. After the styles had remained in the dye for several hours, they were placed on slides, the epidermis was removed, and the styles were then pressed with cover glasses. The chloral hydrate solution containing the stain was used as a mounting medium, and the slides were sealed with a mixture of paraffin and gum mastic.

ABNORMAL DEVELOPMENT OF EMBRYO AND ENDOSPERM

It was found that pollen germinated and grew down the styles in reciprocal crosses of American 26-chromosome \times Asiatic 13-chromosome species. In both crosses pollen tubes entered more than half the embryo sacs.

Embryos initiated development and seemed for a time to develop as fast as or faster than normal embryos. The development of the endosperm started about the usual time; within 4 to 6 days, however, it usually showed evidence of being abnormal.

Material collected 5 days after pollination of a cross with an American 26-chromosome species as female showed that the ovules were smaller and that the endosperm was usually below normal in development and vigor. The ovules were much undersized 7 days after pollination; the endosperm appeared poorly developed and was clumped toward the center of the embryo sac; but the embryos appeared to be about normal in size. Ordinarily, the capsules aborted 8 to 10 days after pollination, but, as will be explained later, it was possible to make a few capsules continue development. At 19 days after pollination some of the embryos were still growing, although they were much below normal in size. Some ovules of this age had a trace of endosperm.

In the reciprocal cross, with Asiatic 13-chromosome species as female, the story differed in that ovules enlarged at a normal rate. The endosperm started vigorous development, but it soon appeared aberrant and ceased to develop after 7 to 9 days. By 15 days after pollination the endosperm had disappeared. The hybrid embryos grew as fast as or faster than normal ones until the endosperm began to disintegrate, at which time their growth rate was greatly reduced. An examination of ovules 30 days after pollination, the age hybrid capsules usually abort, showed that they contained embryos one-half to 2 mm. in length, whereas normal embryos of the same age are about 8 mm. in length. Division figures with 39 chromosomes were found in acetocarmine smears of 30-day-old hybrid embryos. These hybrid embryos ordinarily are poorly differentiated; they usually have two or more thick lobes in place of cotyledons or consist of undifferentiated tissue.

It became evident that, if a method could be found of developing plants from such minute embryos, hybrids could be secured. As the embryos were hardly more than masses of meristematic tissue, a method was tried that was used by White (17) to secure continuous growth of excised tomato roots in culture media made of pure chemical compounds, sucrose, yeast extract, and agar. LaRue (9), with White's methods, was able to produce plants by culturing essentially mature embryos and, with some plants, parts of embryos. By germinating

and culturing undersized hybrid seed on nutrient media, Laibach (8), Jørgensen (7), Skovsted (13), and Tukey (15) were able to produce hybrids that were difficult or impossible to get by the usual methods.

Hybrid embryos, from crosses of female Asiatic 13-chromosome \times male American 26-chromosome species, were dissected from ovules and put on White's culture media after the capsules containing them began to abort. Some of them germinated and formed roots, and the hypocotyls reached a length of about 25 mm., but they had minute deformed structures for cotyledons and never developed further.

RESULTS OF MIXED POLLINATION

METHOD OF POLLINATING

Before the embryology of hybrids between American 26-chromosome and Asiatic 13-chromosome types was studied, it seemed possible that the difficulty in producing the hybrids might result from the fertilization of only a few ovules, which caused the capsules to abort. If this were the trouble, it could be avoided by applying a small amount of pollen from the female plant and then applying an excess of the other type of pollen. Reciprocal mixed pollinations of this type were made.³

The method employed to the greatest extent consisted of using a green-leaf 26-chromosome cotton as female, applying 6 to 12 grains of pollen from another plant of the same species that was homozygous for a dominant anthocyanin factor (red leaf), and then covering the stigmas with an excess of Asiatic 13-chromosome pollen. This method was followed in order that hybrids between the species could be easily detected, for interspecies hybrid seedlings would be green and intra-species hybrids red (3).

PRODUCTION OF HYBRID SEEDLINGS

All the first few seeds germinated gave red seedlings. It was noticed, however, that some seeds failed to germinate, so other seeds were examined for embryos before they were planted. Three seeds of about half-normal size were found in one capsule, and the embryos from these seeds were germinated on White's culture media.⁴ Acetocarmine

Inorganic compound:	(grams per 10,000 cc.)
CaNO ₃	0.98
MgSO ₄38
KNO ₃80
KCl.....	.65
KH ₂ PO ₄125
Fe(SO ₄) ₃025

These compounds are dissolved separately, each in one-thirtieth of the total volume of water (333 cc.) and kept in separate bottles. To make a given amount of media, take four-fifths (of the volume of media) of water, and add one-thirtieth (of the volume of media) of each of the six mineral solutions. To the mixture of the six mineral solutions add 0.6 percent of agar and 2.0 percent of sucrose.

root-tip smears proved them to be hybrids. Further examination of the material produced by mixed pollination showed several minute seeds (fig. 1, *C* and *D*). These seeds were placed on culture media, where several of them germinated (fig. 1, *F* and *G*). They were allowed to grow on the media until they formed roots, and then they

³ Dr. M. M. Rhoades and Dr. J. M. Webber have informed the writer that the method of mixed pollination was previously known to them, and Jørgensen (7) suggested that the application of pollen of a distantly related species to stigmas partly pollinated with pollen of the female parent might be a method of producing haploids.

⁴ An adaptation of White's formula is given here for convenience:

were transferred to pots of sterilized soil. It was necessary to invert beakers over these hybrids until they formed new root hairs. They were watered with distilled water and White's solution until they

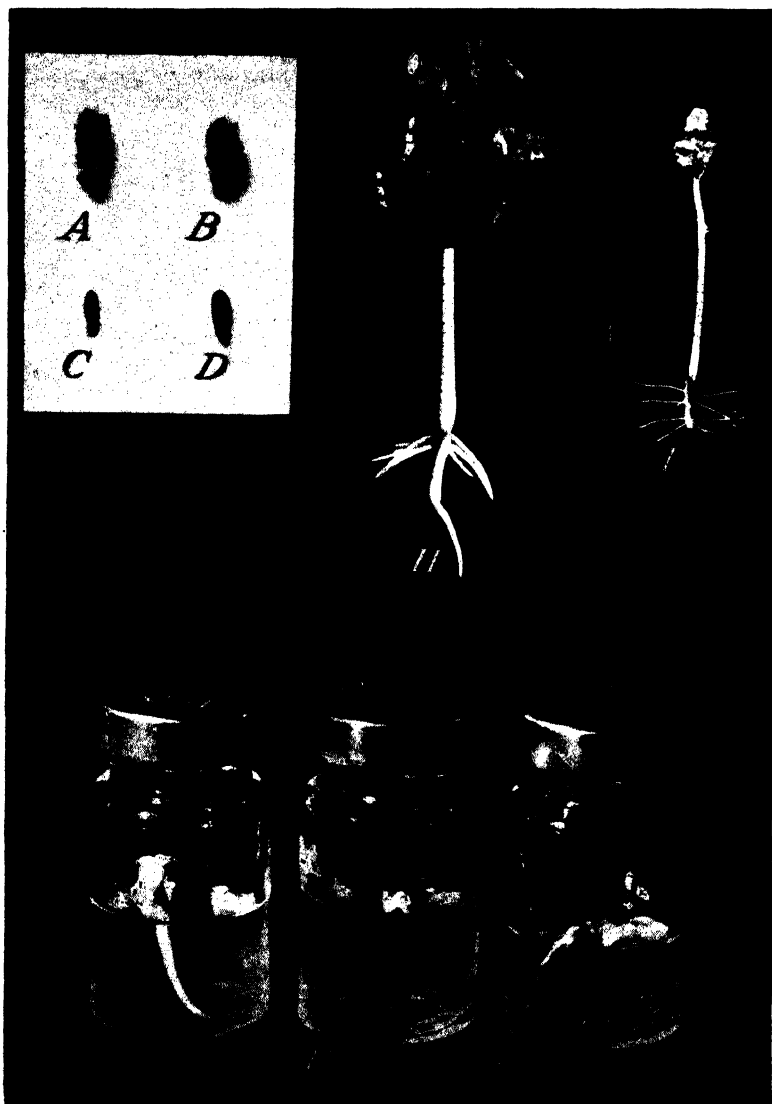


FIGURE 1.—*A* and *B*, Normal seeds of *Gossypium hirsutum*; *C* and *D*, F_1 seeds of *G. hirsutum* \times *G. arboreum* var. *neglectum*, obtained by the method of mixed pollination; *E*, normal seed, and *F* and *G*, hybrid seeds, germinating on culture media; *H*, normal seedling; *I*, comparable hybrid seedling.

showed normal growth. The small size of these hybrids at the time they were transferred to soil is shown by comparing figure 1, *H* and *I*. Skovsted (13) secured hybrids of *G. davidsonii* Kellogg \times *G. sturtii*

F. Muell. "by removing the seed coats, washing in sterile water, and then sowing in sterile cultures of 5 percent dextrose-agar."

By using the method of mixed pollination and germinating the minute embryos on nutrient media, the following *Gossypium* hybrids were produced:

G. hirsutum L. \times *G. herbaceum* L. var. *typicum* Hutchinson and Ghose (6).

G. hirsutum L. \times *G. herbaceum* L. var. *africanum* Hutchinson and Ghose.

G. hirsutum L. \times *G. arboreum* L. var. *neglectum* Watt forma *burmanica* Hutchinson and Ghose.

G. hirsutum L. \times *G. arboreum* L. var. *cernuum* Hutchinson and Ghose.

G. hirsutum L. \times *G. arboreum* L. var. *typicum* forma *bengalensis* Hutchinson and Ghose.

G. barbadense L. \times *G. herbaceum* var. *typicum*.

The apparently normal seeds from about 70 capsules, obtained by the method of mixed pollination, were planted in soil. Two of the seeds produced green plants, while other seeds from the same capsules gave red plants. One of the green plants was a hybrid, the other was a normal American 26-chromosome plant. The natural hybrids reported by Feng (4) and Longley (10) doubtless resulted from mixed pollinations.

One other cross, *G. religiosum* L. \times *G. arboreum* var. *neglectum*, of an American 26-chromosome \times an Asiatic 13-chromosome species was secured from a seed of normal size. In this case, *G. religiosum* was used as female and only pollen of *G. arboreum* var. *neglectum* was used.

If the mixed pollinations are made under optimum conditions more than 75 percent of the capsules reach maturity. Some of them fail to have seeds with hybrid embryos, while others have from one to four minute seeds with hybrid embryos. In the present work hybrids were produced at the rate of one from about each five capsules in which seeds had been produced by the method of mixed pollination.

In relation to the above results of hybridizing American 26-chromosome and Asiatic 13-chromosome cottons, it is of interest to note that an autotetraploid Asiatic cotton with the same number of chromosomes as American cultivated cottons hybridizes easily with American 26-chromosome pollen.

DISCUSSION

Hybrids between cultivated American 26-chromosome and Asiatic 13-chromosome cottons are useful in studying the relationships between the two types and to secure evidence on the origin of the tetraploid American type. Of course only a few hybrids are necessary for such purposes. Recently, however, by the use of colchicine, it has been possible to produce fertile polyploids from the sterile hybrids (1). Since there are numerous varieties of American 26-chromosome and Asiatic 13-chromosome cottons, and since slightly different combinations give polyploids with different characteristics, the reason is obvious for the production of numerous hybrids between the types that involve different combinations of varieties.

It is comparatively easy to produce hybrids of American 26-chromosome \times Asiatic 13-chromosome species by germinating the minute hybrid seeds on sterile culture media. This technique can also be used to secure plants from nonhybrid seeds that ordinarily fail to survive. For example, two normal plants were grown from tiny seed, motes, from crosses that ordinarily give normal seeds.

Doubtless genetic types could be grown, by using the described method, from abortive seeds that would fail to survive under the usual cultural conditions.

Workers have tried various methods in attempts to produce hybrids between American 26-chromosome and Asiatic 13-chromosome cottons. Desai (2) applied substances to the stigma to stimulate pollen germination. The fact that pollen germinates and tubes enter ovules shows that nothing is gained by applying substances to the stigmas. Tanaka (14) tried to make capsules with hybrid embryos remain on plants by ringing and wiring the fruiting branches. The study of the embryology of the hybrids shows that such practices can be of little value.

SUMMARY

In reciprocal crosses of American 26-chromosome \times Asiatic 13-chromosome cottons, the pollen germinates and pollen tubes enter more than half the embryo sacs. Embryo and endosperm development is initiated, but soon becomes aberrant.

By using the American 26-chromosome type as female and a few grains of pollen from a 26-chromosome type along with an excess of Asiatic 13-chromosome pollen, it is possible to produce minute hybrid seeds. Plants can be produced from the seeds by germinating them on sterile culture media. Hybrids involving six combinations of American 26-chromosome \times Asiatic 13-chromosome cottons were produced.

This dependable method of producing hybrids between American and Asiatic cottons will be useful in producing numerous hybrids from which polyploids can be produced.

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A PHYSIOLOGICAL STUDY OF SOFT SCALD IN JONATHAN APPLES ¹

By ERSTON V. MILLER, *physiologist*, and HAROLD A. SCHOMER, *assistant physiologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture* ²

INTRODUCTION

Although soft scald of apples (*Malus sylvestris* Mill.) has been attributed to a deficiency of oxygen in the surrounding air, the various control measures reported in the literature have not been limited to aeration of the stored fruit. Among the means of control may be mentioned placing the fruit in cold storage without delay (2, 8),³ maintaining a storage temperature of 36° F. (11), coating the fruit with paraffin (6) or with a mixture of oil and paraffin (5), and short prestorage exposures to partial vacuum, a high temperature, or carbon dioxide (3).

Brooks and Harley (3) suggested that although the beneficial effects of certain treatments may be due to the elimination of accumulated respiratory products, the effects of "changed metabolism" should not be overlooked. Plagge and Gerhardt (10), writing on soggy breakdown of Grimes Golden apples, had already shown that delayed storage produced changes in metabolism by decreasing the acidity and increasing the specific gravity of the juice. The rate of acetaldehyde and alcohol production has been considered an index of the physiology of stored fruits, and recently these substances have been shown to accumulate in apples affected by soft scald (9, 12).

The present investigation was undertaken for the purpose of obtaining a better understanding of the physiological changes produced in apples by treatments known to influence the incidence of soft scald.

MATERIALS AND METHODS

In 1933 and 1934 Jonathan apples were obtained from near Hancock, Md., and in 1935 from near Frederick, Md. The fruit was transported by motortruck from the orchard to the cold-storage rooms at the Arlington Experiment Farm, Arlington, Va., and placed under the following storage conditions employed for the purpose of studying the development of soft scald: Immediate storage at 32° and 36° F.; holding at 65° to 75° for from 3 to 11 days before storing at 32° and 36°; and subjecting the fruit to heating or to carbon dioxide treatment, following the delay and prior to storage at 32°. A bushel of fruit was reserved in each lot for chemical analyses, and a sample consisting of 25 to 30 fruits was removed at regular intervals.

Sugar determinations were made on duplicate 50-gm. samples of pulp or 25 ml. of juice. Analytical methods were identical with those recommended for plants by the Association of Official Agricultural

¹ Received for publication October 9, 1939.

² The writers are indebted to Charles Brooks and Lacy McColloch, of this Division, for helpful suggestions and assistance in this work.

³ Italic numbers in parentheses refer to Literature Cited, p. 191.

Chemists (1). Sugar was determined either gravimetrically or by the volumetric permanganate method.

Acetaldehyde content of the fruit pulp was determined by a method previously described (7). Hydrogen-ion values of the juice were obtained by means of a calomel half cell, a quinhydrone electrode, and a Leeds and Northrup potentiometer. Total acidity was determined by titrating the juice with N/10 sodium hydroxide to pH 7 by means of the above-mentioned hydrogen-ion apparatus.

The "acid index" was computed according to the method of Du Toit and Reyneke (4). Actually it represents the ratio of the active

acidity to total acidity $\left(\frac{H^+}{H_2^-}\right)$.

Inasmuch as the 3 years' experiments were replications, only data for 1 year are presented when they are considered typical of all 3 years.

RESULTS

ACETALDEHYDE

In figure 1 are curves showing the acetaldehyde content of Jonathan apples stored in 1934. These curves are typical of the effect of delayed storage, and delayed storage followed by carbon dioxide treatment, on the acetaldehyde content. First it will be noted that the fruit placed immediately in 32° F. storage showed a gradual but slight increase in acetaldehyde content during the storage period. A delay of 6 days at 65° prior to storage at 32° increased the acetaldehyde content from 0.1 mg. per 100 gm. of fresh tissue to 0.4 mg. at once, and at about the middle of the storage period the amount was 1.2 mg. A 2-day treatment with 35 percent carbon dioxide in a closed container increased the acetaldehyde content to 1.4 mg., the highest found in this whole lot, but the midstorage value in this particular lot was lower than the others at that time. By January 1935, the end of the storage period, the acetaldehyde content was not high in any lot, but lowest of all in the lot treated with carbon dioxide. There is no apparent relationship between the acetaldehyde content and the development of soft scald. The results for the fruit stored at 36° are similar to those for fruit stored at 32°.

In table 1 the results for the 1935 crop are reported. This table is included not because the results are in disagreement with the others but because the fruit as a whole produced much smaller quantities of acetaldehyde. In lots 1 and 2 stored immediately at 32° and 36° F. the amounts were 0 for all samples. Nevertheless the effects of delayed storage and carbon dioxide treatment are consistent with those obtained previously. Increase in acetaldehyde content as a result of the 3-day delay is not evident until the October sampling, but the effect of the 6-day delay on acetaldehyde content is immediate. The carbon dioxide treatment produced the highest acetaldehyde content of all the lots. An analysis of the data shows that 32° storage is more conducive to incidence of soft scald than 36° and that a delay of more than 3 days increases the amount of soft scald unless the delay is followed by a carbon dioxide treatment. This is in agreement with earlier reports.

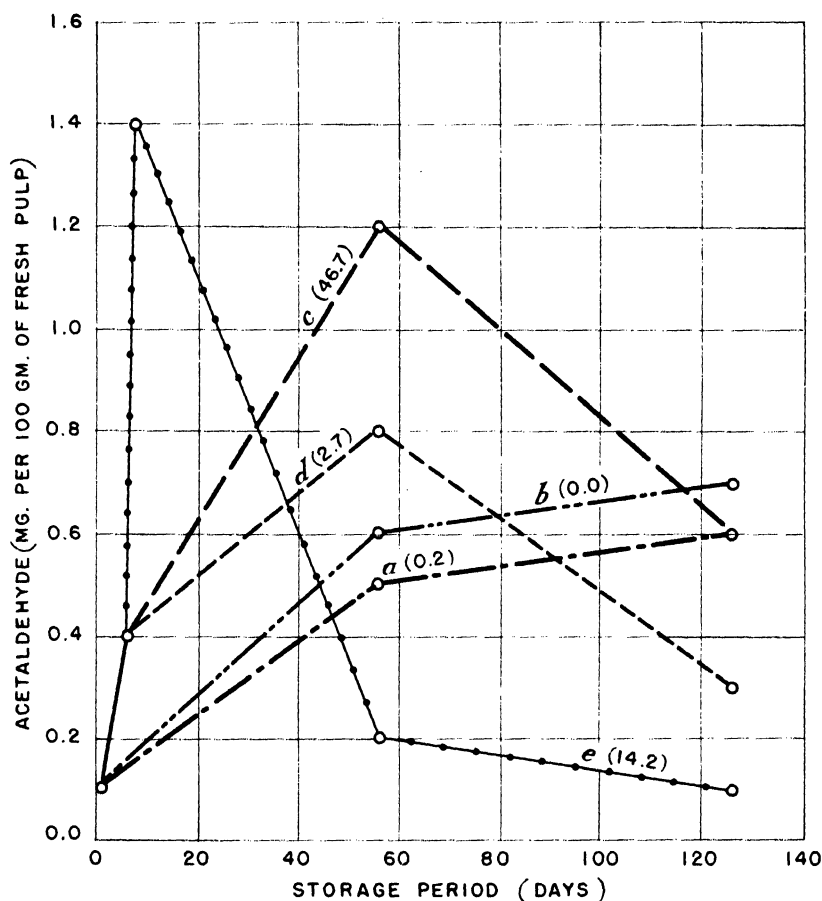


FIGURE 1.—Effect of different storage conditions on the acetaldehyde content of Jonathan apples: *a*, Immediate storage at 32° F.; *b*, immediate storage at 36°; *c*, delayed 6 days at 65°, then stored at 32°; *d*, delayed 6 days at 65°, then stored at 36°; *e*, delayed 6 days at 65°, treated 2 days with 35 percent carbon dioxide, then stored at 32°. Chemical analyses were made in September 1934, at the beginning of the storage period; on November 5; and on January 14, 1935. Numbers on the curves represent percentages of soft scald appearing at the end of the storage period.

TABLE 1.—*Acetaldehyde content of pulp of Jonathan apples at various periods of storage in 1935-36*

Treatment	Acetaldehyde per 100 gm. of fresh pulp						Soft scald at end of storage
	September		October	November	December	January	
	Original sample	After pre-storage treatment					
	Milli-gram	Milli-grams	Milli-gram	Milli-gram	Milli-gram	Milli-gram	Percent
Immediate storage at 32° F.	0		0.0	0.0	0.0	0.0	9.5
Immediate storage at 36°	0		.0	.0	.0	.0	.0
Delayed 3 days at 70°; stored at 32°	0	0.0	.1	.0	.0	.0	3.6
Delayed 3 days at 70°; stored at 36°	0	.0	.1	.1	.0	.1	.0
Delayed 6 days at 70°; stored at 32°	0	.1	.0	.2	.0	.0	32.9
Delayed 6 days at 70°; stored at 36°	0	.1	.0	.1	.1	.0	.6
3 days at 70°; 2 days in 42-50 percent CO ₂ ; stored at 32°	0	.8	.0	.1	.0	.0	.6
6 days at 70°; 2 days in 42-50 percent CO ₂ ; stored at 32°	0	1.9	.0	.1	.1	.0	1.9

SUGAR

The results of the sugar analyses of juice for the 1933-34 and 1934-35 experiments are presented in table 2. In 1935-36 the apple pulp was analyzed. These results appear in table 3.

The prestorage treatment of the 1933 fruit produced no significant changes in the sugar constituents of the juice. By following the sugar content throughout the storage period, it will be seen that the trend of sucrose and total sugar is downward whereas that of reducing sugar is upward. The only lot that stands out from the others is the one that was delayed 11 days at 70° F. and then treated with carbon dioxide before final storage at 32°. This lot had the highest total sugar content throughout the storage period and developed no soft scald.

In the 1934 fruit (table 2) the reducing sugar fluctuated slightly during the storage period, but the trend appeared to be generally upward as in the experiment of the previous year. Total sugar values were somewhat higher at the end than at the beginning of the storage period. Sucrose values showed no consistent trend. Delayed storage appeared to increase the sucrose content of the juice. In these experiments the carbon dioxide treatment had no effect on the sugar content of the juice and there was no apparent relation between sugar changes and development of soft scald.

In the 1935 experiments (table 3) the prestorage treatment produced an immediate increase in sucrose and total sugar with no significant effect on reducing sugar except in lot 7. During the storage life of the fruit the reducing sugar, total sugar, and sucrose fluctuated irregularly. The drop in total sugar that occurred at the October sampling, except in lot 8, is difficult to explain. There was no significant fluctuation in moisture in any of the samples during the whole period. Only lots 1, 3, and 5 showed very high percentages of soft scald, and there was no apparent relation between changes in sugar content and the development of this disorder.

TABLE 2.—*Sugar content of juice of Jonathan apples at various periods of storage in 1933-35*

DATA FOR 1933-34

Treatment	Sugar per 100 ml. of juice										
	September			November			January			Soft scald at end of storage	
	Original sample			After prestorage treatment							
	Reduc- ing sugar	Sucrose	Total sugar	Reduc- ing sugar	Sucrose	Total sugar	Reduc- ing sugar	Sucrose	Total sugar		
Immediate storage at 32° F	Grams 7.62	Grams 3.74	Grams 11.36	Grams 7.69	Grams 3.96	Grams 11.65	Grams 7.88	Grams 3.11	Grams 10.99	Grams 2.61	Percent 31.8
4 days at 70°, then 32°	7.62	3.74	11.36	7.65	3.47	11.12	7.50	3.42	10.92	8.12	31.8
CO ₂ ; then 32°	7.62	3.74	11.36	7.90	3.64	11.54	7.64	3.13	10.77	8.23	47.3
11 days at 70°, then 32° storage	7.62	3.74	11.36	7.95	4.04	11.99	7.62	3.34	10.96	8.29	15.0
CO ₂ ; then 32°	7.62	3.74	11.36				8.05	3.34	11.39	8.13	18.3
										2.33	0
										11.18	

DATA FOR 1934-35

Immediate storage 32° Immediate storage 36° 6 days 65°, then 32° 6 days 65°, then 36° 6 days 65°, 2 days in 35 percent CO ₂ , then 32°	7.95 7.95 7.95 7.95 7.95	3.08 3.08 3.08 3.08 3.08	11.03 11.03 11.03 11.03 11.03	7.54 7.54 7.54 7.84	4.01 4.01 4.01 3.75	11.55 11.55 11.55 11.59	8.40 7.93 7.88 7.85 8.26	3.38 3.46 4.49 4.47 3.61	2.75 3.07 3.10 3.46 2.81	11.25 11.81 11.95 12.27 11.41	0.2 0 46.7 2.7 14.2
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TABLE 3.—Reducing sugar, sucrose, and total sugar content of pulp of Jonathan apples after various periods of storage in 1935-36

Lot No.	Treatment	September						October			November			December			January			Soft scald attend of stor- age
		Original sample			After prestorage treatment			Re- duc- ing sugar	Su- crose	Total sugar	Re- duc- ing sugar	Su- crose	Total sugar	Re- duc- ing sugar	Su- crose	Total sugar				
		Re- duc- ing sugar	Su- crose	Total sugar	Re- duc- ing sugar	Su- crose	Total sugar													
																	Per- cent	Per- cent	Per- cent	
1	Immediate storage at 32° F.	7.87	2.78	10.65	7.87	2.78	10.65	6.41	3.20	9.61	8.44	3.81	12.25	8.19	3.32	11.51	8.37	3.39	11.76	9.5
2	Immediate storage at 36°	7.87	2.78	10.65	7.87	2.78	10.65	6.57	3.20	9.77	8.34	3.75	12.09	8.51	3.80	12.31	6.84	2.81	9.65	0
3	3 days at 75°, then 32° stor- age	7.87	2.78	10.65	7.90	4.10	12.00	6.67	3.23	9.90	8.50	3.89	12.39	8.55	3.24	11.79	6.84	2.66	9.50	3.6
4	3 days at 75°, then 36° stor- age	7.87	2.78	10.65	7.90	4.10	12.00	6.57	3.33	9.90	8.39	3.89	12.28	8.09	3.67	11.76	6.96	2.84	9.80	0
5	6 days at 70°-75°, then 32°	7.87	2.78	10.65	7.84	4.34	12.18	7.45	4.00	11.45	8.19	3.80	11.99	8.47	3.38	11.85	8.26	2.52	10.78	32.9
6	6 days at 70°-75°, then 36°	7.87	2.78	10.65	7.84	4.34	12.18	7.14	3.75	10.89	8.03	3.67	11.70	8.07	3.12	11.19	8.39	2.52	10.91	6
7	3 days at 70°, 2 days in 40 percent CO ₂ ; then 32°	7.87	2.78	10.65	8.20	3.50	11.70	7.56	3.86	11.42	8.12	3.50	11.62	8.00	3.05	11.05	8.30	3.25	11.55	6
8	6 days at 70°-75°, 2 days in 40 percent CO ₂ ; then 32°	7.87	2.78	10.65	7.81	3.73	11.54	8.03	3.82	11.85	8.42	3.56	11.98	8.07	3.19	11.26	8.46	3.14	11.60	1.9

ACIDITY

In table 4 are shown the changes in total acid, pH value, and acid index during the storage period, as well as the total amount of soft scald and internal break-down occurring at the end of the storage period, in the 1933-34 experiments. As was to be expected, the total acid and hydrogen-ion concentration decreased as the period of storage progressed. The treatments involving delayed storage lowered the total acidity at once. In the November sampling the lots treated with carbon dioxide showed a lower acidity than the corresponding untreated lots, but in January this was true only of the lots that had been delayed 11 days prior to carbon dioxide treatment.

TABLE 4.—Total acid, pH value, and acid index of juice of Jonathan apples stored under various conditions in 1933-34

Treatment	September						November			January			Physiological disorders found at end of storage period	
	Original sample			After prestorage treatment			Total acid (as malic per 100 ml. of juice)	pH	Acid index ($\times 10^{-7}$)	Total acid (as malic per 100 ml. of juice)	pH	Acid index ($\times 10^{-7}$)	Soft scald	Internal break-down
	Total acid (as malic per 100 ml. of juice)	pH	Acid index ($\times 10^{-7}$)	Total acid (as malic per 100 ml. of juice)	pH	Acid index ($\times 10^{-7}$)								
Stored immediately at 32°F.	Gm.			Gm.			Gm.			Gm.			Pct.	Pct.
4 days at 70°; then 32°	.759	3.34	40.3	0.598	3.42	42.5	.600	3.41	43.1	.476	3.51	43.4	47.3	.0
4 days at 70°; 2 days in 25-52 percent CO ₂ ; then 32°	.759	3.34	40.3	.609	3.42	41.7	.550	3.46	41.9	.496	3.61	33.0	15.0	1.9
11 days at 70°; then 32°	.759	3.34	40.3	.576	3.44	42.2	.520	3.49	41.2	.474	3.63	33.1	18.3	.0
11 days at 70°; 1 day at 98.6°-104°; then 32°	.759	3.34	40.3	.489	3.57	36.8	.500	3.56	36.8	.352	3.72	36.2	2.8	38.9
11 days at 70°; 3 days in 20-40 percent CO ₂ ; then 32°	.759	3.34	40.3	.575	3.46	40.3	.470	3.51	43.8	.437	3.89	19.7	.0	11.7

The immediate effect of the heat treatment following the delay was to lower the total acidity and hydrogen-ion concentration. In September the heated lot showed less acidity than any other lot. This particular lot was next lowest in acidity at the November sampling and the lowest again in January. The heat treatment seemed to reduce the amount of soft scald but increased the percentage of internal break-down.

No relation between acidity of the apple juice and development of soft scald in the fruit was evident. This is true whether the amount of disease is compared with total acidity at any stage in sampling or with the rate of loss in acidity. The same is true of the hydrogen-ion values. The acid index was determined for each lot at the different sampling periods, but this figure offers no criterion for predicting the incidence of soft scald.

DISCUSSION

In the present investigation soft scald occurred under conditions usually considered conducive to the onset of this disorder; that is, a higher percentage of soft scald was found in the fruit stored immediately at 32° F. than in that stored at 36°, and holding at 65° to 70° prior to storage at both 32° and 36° greatly increased the percentage of soft scald unless the delay was followed by treatment with carbon dioxide. It would seem, then, that chemical analyses of the apples should indicate the manner in which these treatments have affected the physiology of the fruit. First it appears that, in general, delay disrupted the normal evolution of acetaldehyde. The carbon dioxide treatment following the delay always changed the rate of acetaldehyde evolution. The carbon dioxide treatments increased the acetaldehyde at the time of the first sampling following the treatment.

The relation between acetaldehyde content of apples and the incidence of physiological disorders is still not well understood. Although volatile substances such as acetaldehyde and alcohol have been shown to accumulate in small quantities during the normal storage life of apples, they may be increased tremendously by anaerobic conditions to the point where they become toxic to the apples; furthermore, fruits suffering from soft scald, soggy break-down, and internal break-down have yielded, upon analysis, large quantities of acetaldehyde and alcohol. On the other hand, Thomas (12) states that ethyl alcohol and acetaldehyde accumulate in the unhealthy tissues of apples after the incidence of "low-temperature breakdown" and "soft or deep scald" but that these substances do not accumulate in apples or pears stored in air so long as the fruit remains physiologically healthy. In the present investigation no direct correlation was shown to exist between acetaldehyde content at any sampling and the ultimate development of soft scald.

No immediate effect of carbon dioxide on the acids in the juice was noted. Delay at high temperatures naturally reduced the acidity, but this result was not affected by the carbon dioxide treatment immediately following it. In the second sampling, however, the lots treated with carbon dioxide were lower in acid than those that had been delayed without subsequent carbon dioxide treatment. Plagge and Gerhardt (10) have reported that a small amount of soggy break-down is associated with a higher acid loss. It has been shown by several investigators that continued exposure of certain fruits to carbon dioxide lowers the acidity. The data presented here indicate that this effect upon acidity might be carried over for 2 months, yet they are rather meager to justify such a conclusion.

The sugar content of the juice or of the pulp of the apples at any of the sampling times showed no relation to the rate of soft-scald development. Plagge reported that one of the effects of delayed storage was to increase the sugar content of the juice. Hockey and Boyle (7) stated that the amount of sucrose and total sugars present in Gravensteins at the time of picking had little or no effect on the prevalence of "spot scald." In the present work the total sugar values at the time of the last sampling indicate a similarity between the delayed lots treated with carbon dioxide and those stored immediately at 32° F. It might be inferred that the changes in metabolism produced by the delayed storage were counteracted by the subsequent carbon

dioxide treatment. The results on soft scald suggest this also. A larger percentage of soft scald occurred in the delayed lots unless the delay was followed by treatment with carbon dioxide.

That the sugar, acid, acetaldehyde, and alcohol content of apples constitutes an index of the metabolic activity is suggested by the changes brought about by the various storage treatments, but no evidence has been found that these substances are determining factors in the development of soft scald.

SUMMARY

A physiological study was made of Jonathan apples stored under conditions known to influence the incidence of soft scald. These conditions consisted of immediate storage at 32° and 36° F., delays of 3 to 11 days at 65° to 75° prior to storage at the above-mentioned temperatures, and a carbon dioxide or a heat treatment following the delay but prior to storage at 32° and 36°.

Fruit stored immediately generally evolved acetaldehyde in small quantities but in slowly increasing amounts, whereas a delay at 65° to 75° F. greatly increased the amount of acetaldehyde during the early part of the storage life of the fruit. Treatment with carbon dioxide following the delay increased the acetaldehyde content of the fruit.

Delay at high temperatures (65° to 75° F.) tended to increase the sucrose and total sugar content of both juice and pulp samples at the beginning of the storage period. Treatment with carbon dioxide did not greatly influence reducing sugar and sucrose.

Delay reduced the acidity of the juice at the beginning of storage, and carbon dioxide treatment had no immediate additional effect. The lots treated with carbon dioxide showed a slightly lower acidity at the November sampling, which was approximately the middle of the storage period.

Heating the fruit to 98.6°–104° F. for 1 day following an 11-day delay at 70° lowered the total acidity and hydrogen-ion concentration. This lot of fruit showed less soft scald but more internal break-down than the other lots.

Although changes in sugar, acid, and acetaldehyde content of apples are doubtless an index of physiological activity, no correlation was found between these substances and the occurrence of soft scald.

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A CYTOLOGICAL STUDY OF SOME SPECIES IN THE GENUS *PASPALUM*¹

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INTRODUCTION

The genus *Paspalum* L. belongs to the large tribe Paniceae in the grass family, the Gramineae. With the exception of *Paspalum bosci-anum* Flüggé, the 42 species occurring quite commonly in the south-eastern part of the United States are perennials (5, pp. 575-601).³ Many of these species make a valuable contribution to the native grass pasture in the Southeast, and *P. dilatatum* Poir., *P. notatum* Flüggé, and *P. urvillei* Steud. are showing much promise in some of the improved pastures in this region.

A search of the literature reveals that only five species in this genus have been studied cytologically, and, with the exception of *Paspalum dilatatum*, those studied are of little importance in the Gulf States. The results of these studies, by Avdulov (1, 2), Avdulov and Titova (3), Church (4), and Marchal (8), are presented in table 1.

The failure to find resistance to ergot (*Claviceps paspali* Stevens and Hall) in *Paspalum dilatatum* and several other promising species suggested the wisdom of resorting to species hybridization for the improvement of these grasses. It was with a desire to arrive at a better understanding of the cytology of the *Paspalum* species, and hence a clue as to which hybrid combinations might be expected to be most fertile, that this study was begun.

TABLE 1.— Somatic chromosome numbers in *Paspalum* species as reported in the literature

Species	Somatic chromosome number (2n)	Reference
<i>P. dilatatum</i> Poir.	1 40	(8)
<i>P. pubescens</i> Muhl.	1 20	(4)
<i>P. scrobiculatum</i> L.	40	(7)
<i>P. stoloniferum</i> Bose.	1 20, 20, 23	(8, 3)
<i>P. virgatum</i> L.	80	(2)

¹ Reported as the *n* number; the number here given is double that reported.

MATERIALS AND METHODS

All plant material used in this study was grown either in the grass introduction garden or on the premises of the Georgia Coastal Plain Experiment Station, Tifton, Ga. Since many obstacles were encoun-

¹ Received for publication December 1, 1939. Cooperative investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, the Georgia Experiment Station, and the Georgia Coastal Plain Experiment Station.

² The writer wishes to acknowledge the assistance of E. J. O'Neal, agent, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in the preparation of material for cytological study.

³ Italic numbers in parentheses refer to Literature Cited, p. 197.

tered in the use of the acetocarmine technique, all studies reported here were made on root-tip material. Root tips collected between 8 and 10 a. m. were fixed in Craf fixative for 24 hours. The embedding procedure consisted of a slight modification of the alcohol-chloroform method as revised by La Cour (6). Sections were cut 10μ thick and

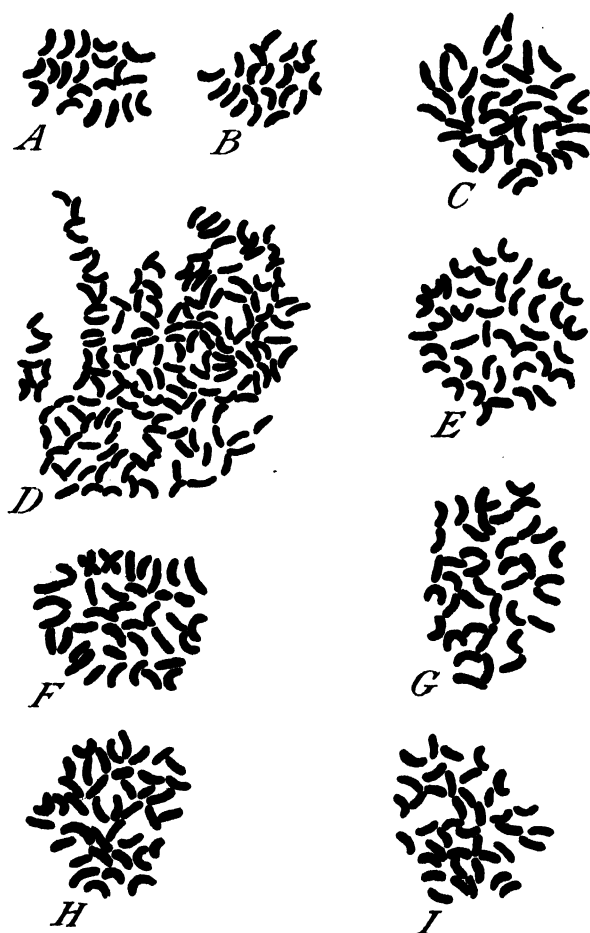


FIGURE 1.—Camera lucida drawings of chromosomes on equatorial plates in cells from root tips of nine species of *Paspalum*: A, *P. ciliatifolium*, $2n=20$; B, *P. paniculatum*, P. E. I. 128190, $2n=20$; C, *P. dilatatum*, $2n=40$; D, *P. floridanum*, $2n=160$; E, *P. urvillei*, $2n=40$; F, *P. malacophyllum*, F. C. 04240, $2n=40$; G, *P. boscianum*, $2n=40$; H, *P. notatum*, $2n=40$; I, *P. notatum*, P. E. I. 121415, $2n=40$.

were stained by the iodine-gentian violet technique described by La Cour (6). With the exception of *Paspalum floridanum* Michx., all chromosome numbers were determined by making camera lucida drawings of three to five different equatorial plates in each species. Although much material of *P. floridanum* was examined, only in the equatorial plate shown in figures 1, D, and 2, A, were the chromosomes well enough separated to permit a count.

OBSERVATIONS

A list of the *Paspalum* species examined, together with their somatic chromosome numbers, is presented as table 2. The camera lucida drawings and photomicrographs from which chromosome numbers were determined are presented as figures 1 and 2. A brief description of each species follows.

TABLE 2.—Somatic chromosome numbers in the *Paspalum* species examined at Tifton, Ga.

Text figure No.	Species	Somatic chromosome number (2n)	Text figure No.	Species	Somatic chromosome number (2n)
1, A; 2, B	<i>P. ciliatifolium</i>	20	1, F	<i>P. malacophyllum</i> , F. C. 04240.	40
1, B	<i>P. paniculatum</i> , P. E. I. 128190.	20	1, G	<i>P. boscianum</i>	40
1, C	<i>P. dilatatum</i>	40	1, H	<i>P. notatum</i>	40
1, D; 2, A	<i>P. floridanum</i>	160	1, I	<i>P. notatum</i> , P. E. I. 121415	40
1, E	<i>P. urvillei</i>	40			

Paspalum ciliatifolium Michx.—This species, a native of the southeastern part of the United States, occurs in cleared or semicleared areas and supplies some of the best grazing to be found in many native pastures in Georgia and Florida. Since the data presented in tables 1 and 2 indicate that 10 is the basic chromosome number in this genus, the plants examined here may be considered diploid, $2n=20$ (figs. 1, A; 2, B).

Paspalum paniculatum, L., P. E. I.⁴ 128190.—Seed of this species was sent to the United States Department of Agriculture from Navua, Fiji Islands, by Richard B. Howard in 1938. The grass has made a luxuriant growth in the greenhouse and is being tested in the introduction garden this year. This species proved to be diploid, $2n=20$ (fig. 1, B).

Paspalum dilatatum Poir.—Most widely used of all *Paspalum* species in improved pastures in the Gulf States, this grass was introduced into the Southern States from Uruguay or Argentina about the middle of the last century (5). The scarcity and cost of high-quality Dallis grass seed, a result of its susceptibility to ergot and its dependence upon a relatively high soil-nutrient level for satisfactory growth, are the principal factors limiting its use.

The material examined was tetraploid, $2n=40$ (fig. 1, C). This agrees with the n number reported by Marchal (8).

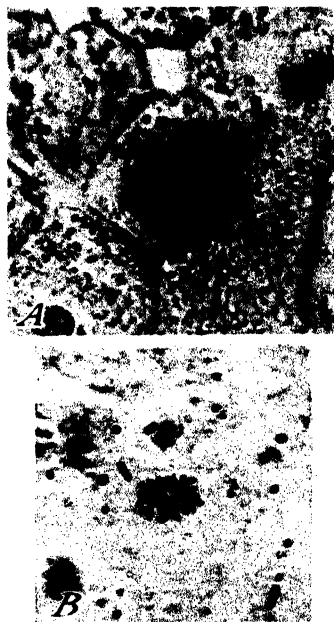


FIGURE 2.—Photomicrograph of equatorial plate in root tip of (A) *Paspalum floridanum* ($\times 700$) and (B) *P. ciliatifolium* ($\times 850$).

⁴ Refers to accession number of the Division of Plant Exploration and Introduction.

Paspalum floridanum Michx.—This grass is one of the large-seeded, robust species of *Paspalum*, occurring in low, moist, sandy soil, flat woods, pine woods, and low prairies throughout most of the Coastal Plain south of Maryland (5). The huge ergots formed in the spikelets of this species demonstrate in a striking manner that this grass is not a source of genes for ergot resistance. Although only one figure could be found which was suitable for counting, the material examined, together with the apparent polyploid nature of the genus, leaves little doubt that this species is sixteenploid, $2n=160$ (figs. 1, D; 2, A).

Paspalum urvillei Steud.—This erect bunch grass grows along ditches and roadsides and in moist and wet soils in most of the Southeastern States. Its heavy seed production, tolerance of poorly drained soils, and ability to make good growth on soils of low fertility are desirable features of this grass. Its failure to withstand close grazing, probably due to its upright growth habit, usually causes it to "go out" in improved pastures in this region. Cytologically it was found to be tetraploid, $2n=40$ (fig. 1, E).

Paspalum malacophyllum Trin., F. C. 04240.—This species, introduced from Argentina a number of years ago, has been receiving considerable attention during the past few years. Although evidence is available that this erect bunch grass will go out when heavily grazed, its vigor, adaptability, and heavy seed production make it one of the most promising grass introductions under observation at Tifton. The failure of the writer to find ergot sclerotia on this grass when growing in association with heavily infected Dallis grass during the last 3 years at Tifton and the failure of Lefobvre (7) to obtain ergot infection upon inoculation, indicate that it is the best source of genes for ergot resistance now available. The material examined was found to be tetraploid, $2n=40$ (fig. 1, F).

Paspalum boscianum Flüge.—This rather succulent annual, found growing in moist or wet fields and along ditches and ponds in most of the Coastal Plain, proved to be tetraploid, $2n=40$ (fig. 1, G).

Paspalum notatum Flüge.—This species, introduced from the West Indies, Mexico, and parts of South America, is showing much promise as a permanent pasture grass on the sandy soils of Georgia and Florida. Productivity, extensive root system, strong feeding power, stoloniferous habit, and ability to withstand close grazing are outstanding features of this species. Plants which may be referred to as common Bahia grass were tetraploid, $2n=40$ (fig. 1, H).

Paspalum notatum Flüge, P. E. I. 121415.—Early in 1937, W. A. Archer sent to Washington, D. C., a packet of seed from Santissima, Trinidad, Paraguay, which was identified as *Paspalum notatum*. Not only were the seeds smaller than common Bahia grass, but the plants which grew from them differed so much from common Bahia that there was some question as to whether this might not be a different species. Its greater frost resistance and its ability to grow at lower temperatures than common Bahia grass were promising features of this introduction. Cytologically this grass was found to be tetraploid, $2n=40$ (fig. 1, I). The fertility of the hybrids with common Bahia grass produced in 1938 and of plants grown from seed in 1939 should help to explain the taxonomy of this grass.

RELATION BETWEEN AREA OF EQUATORIAL PLATE AND NUMBER OF CHROMOSOMES

The relationship between the area of the equatorial plate, calculated from average diameter measurements of the plates in figure 1, *A* to *I*, and the number of chromosomes in the plate is of interest. In the nine *Paspalum* species considered, the average calculated equatorial plate area for the diploid species was 180 mm.²; for the tetraploid species, 415 mm.²; and for *Paspalum floridanum*, the sixteenploid species, 1,451 mm.² If the area of the equatorial plate were directly proportional to the chromosome number of the species, these areas would be 180, 360, and 1,440 mm.², respectively. Thus it is apparent that a positive correlation of rather high magnitude exists between equatorial plate area and chromosome number in the *Paspalum* species examined in this study.

SUMMARY AND CONCLUSIONS

With evidence that 10 is the basic (*n*) number of chromosomes the 12 *Paspalum* species considered fall into a polyploid series ranging from 2*n* to 16*n*. In this group there are 4 diploids, 6 tetraploids, 1 octoploid, and 1 sixteenploid. *P. ciliatifolium*, *P. paniculatum*, *P. stoloniferum*, and *P. pubescens* are diploid; *P. dilatatum*, *P. notatum*, *P. malacophyllum*, *P. urvillei*, *P. boscianum*, and *P. scrobiculatum* are tetraploid; *P. virgatum* is octoploid; and *P. floridanum* is sixteenploid. The fact that all of the most promising pasture species are tetraploids is worthy of note. The rather close correlation that exists between the calculated areas of the equatorial plates in figure 1, *A* to *I*, and their chromosome numbers is interesting.

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CHEMICAL EFFECT ON LEAD ARSENATE OF CERTAIN SALTS WHICH MAY BE PRESENT IN SOIL AND SPRAY WATERS¹

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INTRODUCTION

The present practice by orchardists of applying several lead arsenate sprays during the growing season leads to a gradual deposition of appreciable quantities of arsenic on the trees. Most of the arsenic thus applied finds its way sooner or later to the soil where it may accumulate year after year, especially in soils where drainage is poor. Moreover, the recent tendency to apply arsenicals directly to the soil (11)² in comparatively large quantities in order to kill the larvae of Japanese beetle (*Popillia japonica* Newm.) and other grubs still further enhances the possibility of accumulating large quantities of arsenic in certain soils.

As long as the arsenic compound remains insoluble on the plant or in the soil, there is no danger of injury to fruit trees or crops. However, as soon as the lead arsenate comes in contact with water or water containing soluble salts, especially alkalies, chemical reactions may take place by which appreciable quantities of water-soluble arsenic may be released and become available for absorption by the plant roots. This so-called "soluble arsenic" may be in the form either of arsenic acid (H_3AsO_4) or water-soluble salts such as $\text{K}_2\text{H AsO}_4$, Na_2HAsO_4 , $(\text{NH}_4)_2\text{HAsO}_4$, depending on the kinds of bases, capable of displacing lead from the PbHAsO_4 molecule, present in soil solution.

In addition to the salts present in soil, waters used for spraying insecticides, especially hard waters, in many instances contain appreciable quantities of salts such as sulfates, chlorides, carbonates, bicarbonates, and nitrates, which may react directly with lead arsenate, forming soluble arsenic.

It has been definitely established by Kilgore (10), Headden (9), Clinton and Britton (4), Smith (16), Swingle, Morris, and Burke (17), Ginsburg (6, 7, 8), and other investigators that arsenical injury to plants may be caused by soluble arsenic either originally present in the spray mixture or subsequently formed. That small quantities of soluble arsenic present in certain soils may prove injurious to fruit trees and other crops has been shown by Headden (9), Newton (13), Morris and Swingle (12), and others. Cooper and his associates (5) have found that the accumulation of arsenic in certain soils causes a marked reduction in the yield of certain crops. Reed and Sturgis (14) report difficulties encountered by farmers of south-west Louisiana in growing rice following cotton that has been dusted

¹ Received for publication September 2, 1939. Paper of the Journal Series, New Jersey Agricultural Experiment Station, Department of Entomology.

² Italic numbers in parentheses refer to Literature Cited, p. 205.

with calcium arsenate. Carter (2) reports hydrochloric acid, sodium silicate, and sodium hydroxide as the best solvents for the removal of lead arsenate residue from fruits.

Any chemical possessing the properties of decomposing lead arsenate in the spray mixture or in the soil is of immediate importance to the agriculturist, particularly the fruit grower. He is primarily interested to know the chemicals possessing such properties. The purpose of the investigation reported in this paper was, therefore, to determine which of the chemicals normally found in soils or spray waters react with lead arsenate to form soluble arsenic.

MATERIALS AND METHODS

Of the various compounds that one would reasonably expect to find in soils and spray waters, as indicated by Clarke (3), Russell (15), and Weir (18), some 50 outstanding salts were selected for testing. They consisted of several members of each of the following 10 groups: Phosphates, carbonates, bicarbonates, acetates, sulfates, nitrates, chlorides, silicates, sulfides, and sulfites. Chemically pure salts were used in all tests.

In order to determine the amount of soluble arsenic that each of these salts can form from lead arsenate, the following method was employed: A standard brand of acid lead arsenate was mixed at the rate of 2 gm. in 500 cc. of distilled water (approximately 3 pounds per 100 gallons) with approximate stoichiometrical proportions of each salt. The mixture was allowed to stand at room temperature for about 24 hours with frequent shaking. At the end of this period the mixtures were filtered and the soluble arsenic in the filtrate was determined as As_2O_3 by the standard iodine method as outlined in the official method (1). From the percent of As_2O_3 thus found the approximate percent of $PbHAsO_4$ decomposed was then calculated.

Approximate stoichiometrical proportions were employed in order to furnish a uniform basis for comparison. Since $PbHAsO_4$ has a higher molecular weight (347) than any one of the salts tested, the reacting concentrations of the salts were necessarily lower than that of lead arsenate, ranging from 0.084 percent for CaS to 0.258 percent for $BaSO_4$ as compared with 0.4 percent for $PbHAsO_4$. The stoichiometrical concentration of each salt varied according to its molecular weight as well as to its reactive chemical equivalent in regard to $PbHAsO_4$. The concentration of lead arsenate remained constant in all the tests.

In another series of tests the concentration of certain selected salts was increased to several times their stoichiometrical proportions, the lead arsenate remaining constant, and the soluble arsenic formed was again analyzed. The actual concentrations of the salts tested in the two series are given in the tables.

The procedure of preparing the samples for analysis was as follows: The required amount of each salt, based on its anhydrous content, was accurately weighed into a 1-liter flask and mixed with 500 cc. of distilled water. After a few minutes shaking the hydrogen-ion concentration of the solution was determined potentiometrically, by employing the hydrogen electrode. The lead arsenate was then added. The hydrogen-ion concentration was determined to ascertain whether or not the pH value of the solution, by itself, plays any part in decom-

posing lead arsenate. Duplicate samples were run for each analysis. Blanks consisting of the salt in the same concentration, but without lead arsenate, were also run. The averages of the analyses are presented in condensed form in the two tables.

RESULTS AND DISCUSSION

DECOMPOSITION OF LEAD ARSENATE

A comparison of the data in table 1, where the results from all the salts tested are presented, shows that the sulfates, nitrates, and acetates, a total of 19 salts, did not appreciably affect the decomposition of lead arsenate, as may be seen from the low percentages of As_2O_3 analyzed in the filtrates. This disclosure was rather surprising, as all the salts in these 3 groups, except BaSO_4 , are water soluble. The next 5 groups of salts caused the following percentages of decomposition of lead arsenate: Chlorides about 5; silicates from 7.7 to 9.6; carbonates from 4.3 to 47; bicarbonates about 7; and sulfites from 1.4 to 17.8. The highest percent decomposition of PbHAsO_4 occurred in the presence of sulfides, ranging from 30 for CaS to 89.2 for Na_2S_5 .

Most interesting results were obtained with the group of phosphates. The three phosphates of calcium caused no material increases in the decomposition of PbHAsO_4 , as compared with the check where lead arsenate alone was used. Of the three phosphates of potassium, the tribasic (K_3PO_4) decomposed 35; the dibasic (K_2HPO_4) 17.8; and the monobasic (KH_2PO_4) only 1 percent of the lead arsenate. Almost identical results were secured with the corresponding phosphates of sodium (tests 47, 48, 49). In each of these two groups of alkali phosphates the percent of soluble arsenic formed was in direct proportion to the number of either sodium or potassium atoms and in inverse proportion to the number of hydrogen atoms present in the molecule.

EFFECT OF HYDROGEN-ION CONCENTRATION

Since the pH values of the salt solutions tested lie within a wide range, varying from 3.6 for $\text{Ca}(\text{H}_2\text{PO}_4)_2$ to 11.4 for Na_2S_5 , the relationship was studied between the hydrogen-ion concentration of the salt and the percentage decomposition of PbHAsO_4 . A close analysis of the results in table 1 shows that in the majority of cases salts with pH values of 8 or higher formed large amounts of soluble arsenic. On the other hand, salts with pH values of 7 or lower in most instances either did not affect the lead arsenate at all or only slightly increased its solubility. Thus of 19 salts ranging in pH values from 8 to 11.4, 17 decomposed from 7 to 89.2 percent of lead arsenate, while only 2, namely $\text{Na}_2\text{S}_2\text{O}_3$ and CaSO_3 , with pH values of 8.7 and 9.3, respectively (tests 9, 36), did not increase considerably the decomposition of lead arsenate as compared with the check. Again, of 29 salts with pH values from 3.6 to 7.2, 23 decomposed only from 0.28 to 1.5 percent of PbHAsO_4 . Here the 5 chlorides and 2 carbonates tested offer exceptions. Although the pH values of the chlorides ranged from 6.5 to 7.1, the percentages of lead arsenate decomposed were appreciable, varying from 4.73 to 4.98. The carbonates of Ca and Mg (pH 6.5 and 6.4) decomposed 4.3 and 8.26 percent of PbHAsO_4 , respectively.

It should be noted, however, that these results were obtained with chemically pure salts in distilled water. Somewhat different reactions

might be obtained if the same salts were present in soils varying in pH values. Deviations from these results should also be expected in soils high in organic constituents, which may act as buffers in preventing changes in the hydrogen-ion concentration.

TABLE 1.—Soluble arsenic formed from lead arsenate (2 gm. in 500 cc.) when mixed with different salts in approximate stoichiometrical proportions

Test No.	Name of salt	Chemical formula of salt	Approximate solubility of salt in water	Salt tested	pH of salt solution	As ₂ O ₃ produced	PbHAsO ₄ decomposed
			Percent	Percent	Percent	Percent	Percent
1	Aluminum sulfate.....	Al ₂ (SO ₄) ₃	31.00	0.12	3.9	0.40	1.40
2	Ammonium sulfate.....	(NH ₄) ₂ SO ₄	71.00	.153	6.3	.13	.45
3	Barium sulfate.....	BaSO ₄	Trace	.258	5.8	.15	.52
4	Calcium sulfate.....	CaSO ₄18	.136	7.1	.14	.49
5	Copper sulfate.....	CuSO ₄	20.00	.108	4.6	.17	.50
6	Magnesium sulfate.....	MgSO ₄	26.00	.14	6.8	.11	.38
7	Potassium sulfate.....	K ₂ SO ₄	8.50	.20	7.1	.15	.52
8	Sodium sulfate.....	Na ₂ SO ₄	5.00	.165	6.9	.24	.84
9	Sodium thiosulfate.....	Na ₂ S ₂ O ₃	74.00	.184	8.7	.30	1.05
10	Zinc sulfate.....	ZnSO ₄	80.00	.196	6.5	.08	.28
11	Ammonium nitrate.....	NH ₄ NO ₃	(²)	.184	5.7	.19	.66
12	Calcium nitrate.....	Ca(NO ₃) ₂	(²)	.188	5.7	.21	.73
13	Magnesium nitrate.....	Mg(NO ₃) ₂	(²)	.172	7.8	.24	.84
14	Potassium nitrate.....	KNO ₃	13.00	.232	5.8	.35	1.22
15	Sodium nitrate.....	NaNO ₃	73.00	.196	6.5	.39	1.37
16	Calcium acetate.....	Ca(C ₂ H ₃ O ₂) ₂	43.00	.108	6.7	.25	.87
17	Magnesium acetate.....	Mg(C ₂ H ₃ O ₂) ₂	(³)	.152	6.9	.50	1.72
18	Potassium acetate.....	KC ₂ H ₃ O ₂	(²)	.208	6.3	.31	1.09
19	Sodium acetate.....	NaC ₂ H ₃ O ₂	26.00	.176	6.9	.30	1.05
20	Ammonium chloride.....	NH ₄ Cl.....	29.00	.12	6.6	1.40	4.90
21	Calcium chloride.....	CaCl ₂	59.00	.128	6.6	1.35	4.73
22	Magnesium chloride.....	MgCl ₂	52.00	.108	6.5	1.72	4.98
23	Potassium chloride.....	KCl.....	28.00	.172	7.2	1.42	4.96
24	Sodium chloride.....	NaCl.....	35.00	.136	7.1	1.40	4.90
25	Magnesium silicate.....	MgSiO ₃	(⁴)	.108	8.8	2.20	7.70
26	Potassium silicate.....	K ₂ SiO ₃	(³)	.165	9.9	2.25	7.87
27	Sodium silicate.....	Na ₂ SiO ₃	(³)	.128	10.2	2.75	9.63
28	Ammonium bicarbonate.....	NH ₄ HCO ₃	12.00	.092	8.0	2.00	7.00
29	Potassium bicarbonate.....	KHCO ₃	25.00	.110	8.2	2.15	7.55
30	Sodium bicarbonate.....	NaHCO ₃	5.00	.096	8.1	2.11	7.38
31	Ammonium carbonate.....	(NH ₄) ₂ CO ₃	100.00	.11	8.9	5.80	20.30
32	Calcium carbonate.....	CaCO ₃01	.116	6.5	1.20	4.30
33	Magnesium carbonate.....	MgCO ₃01	.096	6.4	2.36	8.26
34	Potassium carbonate.....	K ₂ CO ₃	59.00	.16	10.5	13.40	47.00
35	Sodium carbonate.....	Na ₂ CO ₃	7.00	.12	10.7	13.40	47.00
36	Calcium sulfite.....	CaSO ₃125	.14	9.3	.40	1.40
37	Sodium sulfite.....	Na ₂ SO ₃	28.00	.144	10.0	5.10	17.80
38	Calcium sulfide.....	CaS.....	(⁵)	.084	10.1	8.60	30.00
39	Sodium sulfide, mono.....	Na ₂ S.....	15.00	.09	11.1	25.00	87.50
40	Sodium sulfide, penta.....	Na ₂ S ₅	(⁵)	.24	11.4	25.00	89.20
41	Monocalcium phosphate.....	Ca(H ₂ PO ₄) ₂	(⁴)	.092	3.6	.31	1.10
42	Dicalcium phosphate.....	CaHPO ₄02	.104	7.1	.57	2.00
43	Tricalcium phosphate.....	Ca ₃ (PO ₄) ₂	(⁵)	.12	6.9	.43	1.50
44	Monopotassium phosphate.....	KH ₂ PO ₄	25.00	.104	5.5	.29	1.00
45	Dipotassium phosphate.....	K ₂ HPO ₄	(²)	.132	8.5	5.10	17.80
46	Tripotassium phosphate.....	K ₃ PO ₄	(³)	.162	9.5	10.00	35.00
47	Monosodium phosphate.....	NaH ₂ PO ₄	(²)	.092	5.6	.31	1.10
48	Disodium phosphate.....	Na ₂ HPO ₄	6.00	.112	8.5	5.40	18.90
49	Trisodium phosphate.....	Na ₃ PO ₄	28.00	.128	9.8	9.00	31.50
Check	Lead arsonate only.....				5.2	.18	.63

¹ When exact percentages are not known the following expressions are used: Soluble, slightly soluble, very soluble, decomposes.

² Very soluble.

³ Soluble.

⁴ Decomposes.

⁵ Slightly soluble.

SOLUBILITY OF CHEMICALS

Of all the chemicals tested, only four, Ca₃PO₄, BaSO₄, CaCO₃, and MgCO₃, were not sufficiently soluble at room temperatures to dissolve completely in the volume of water used in the tests. The other com-

pounds were appreciably soluble, many of them very soluble, as indicated in table 1. If the degree of solubility alone is taken as a criterion for measuring decomposition of lead arsenate, there seems to be no direct relationship. Thus MgCO_3 , 0.01-percent soluble, decomposed 8.26 percent, whereas MgSO_4 , 26-percent soluble, decomposed only 0.38 percent of PbHAsO_4 . Again, NaNO_3 and $\text{NaC}_2\text{H}_3\text{O}_2$, both very soluble, decomposed only 1.37 and 1.05 percent of lead arsenate, respectively. An outstanding example is offered by the phosphates. The K_3PO_4 , of slight solubility, decomposed 35 percent, while KH_2PO_4 , 25-percent soluble, decomposed only 1 percent of lead arsenate.

Apparently other factors, in addition to solubility, such as acidity or alkalinity, degree of ionic dissociation, position of the base of the salt in the "displacement series" with respect to lead, and solubility of the reaction products play their parts in determining the extent of reaction of the salt with lead arsenate.

INCREASED SALT CONCENTRATIONS

In seeking more information to explain adequately why some soluble salts, as well as several entire groups of salts, did not react with lead arsenate, it occurred to the writer that the small concentrations used might have been insufficient for the chemical reaction of these salts to take place. Accordingly, another series of tests was run with higher concentrations, using multiples of the stoichiometrical proportions of the salts, theoretically required to react with 2 gm. of acid lead arsenate. For these tests the potassium and magnesium salts of the three groups, nitrates, acetates, and sulfates, as well as the entire group of phosphates, were selected. The actual concentrations of the salts and the soluble arsenic formed are given in table 2.

TABLE 2. Soluble arsenic formed from lead arsenate (2 gm. in 500 cc.) when mixed with different salts in multiples of their approximate stoichiometrical proportions

Test No.	Name of salt	Chemical formula of salt	Salt tested	Multiples of stoichiometrical proportions used	As ₂ O ₃ formed	PbHAsO ₄ decomposed
			Percent		Percent	Percent
51	Potassium sulfate.....	K ₂ SO ₄	5.0	25.0	0.44	1.54
52	Magnesium sulfate.....	MgSO ₄	5.0	35.7	.26	.91
53	Potassium nitrate.....	KNO ₃	1.0	4.3	.36	1.26
54	Magnesium nitrate.....	Mg(NO ₃) ₂	1.0	5.8	.31	1.08
55	Potassium acetate.....	KC ₂ H ₃ O ₂	5.0	24.0	.29	1.02
56	Magnesium acetate.....	Mg(C ₂ H ₃ O ₂) ₂4	2.6	.23	.81
57	Monocalcium phosphate.....	Ca(H ₂ PO ₄) ₂4	4.3	.86	3.01
58	Dicalcium phosphate.....	CaHPO ₄4	3.8	.46	1.60
59	Tricalcium phosphate.....	Ca ₃ (PO ₄) ₂4	3.3	.45	1.57
60	Monopotassium phosphate.....	KH ₂ PO ₄4	3.8	.25	.87
61	Monosodium phosphate.....	NaH ₂ PO ₄4	4.4	.40	1.40
62	Dipotassium phosphate.....	K ₂ HPO ₄4	3.0	7.30	25.55
63	Disodium phosphate.....	Na ₂ HPO ₄4	3.6	9.60	33.60
64	Tripotassium phosphate.....	K ₃ PO ₄4	2.4	16.50	67.75
65	Trisodium phosphate.....	Na ₃ PO ₄4	3.1	15.10	52.85
66						
Check	Lead arsenate only.....		.4	1.0	.22	.77

The results from the second series substantiate the first findings, namely, that nitrates, sulfates, acetates, and monobasic phosphates do not form soluble arsenic from PbHAsO_4 , regardless of the concentrations employed. Thus, increasing the concentrations of K_2SO_4 and $\text{KC}_2\text{H}_3\text{O}_2$ to 5 percent, or about 24 times the amount previously used, did not appreciably change the low percentages of soluble arsenic found in the filtrates. Similar results were also shown by the phosphates. Increasing the quantities of NaH_2PO_4 and KH_2PO_4 to about 4 times the concentration used in the first series of tests, produced only 0.4 percent and 0.25 percent of As_2O_3 , respectively. Of the 3 calcium phosphates, only $\text{Ca}(\text{H}_2\text{PO}_4)_2$ showed a small increase of soluble arsenic, giving 0.86 percent (test 57) of As_2O_3 as compared with 0.31 percent (test 41).

On the other hand, the dibasic and the tribasic phosphates of sodium and potassium, which formed large amounts of soluble arsenic in the first series of tests, still further increased the percentages of As_2O_3 in the filtrates of the second series, as may be seen by comparing tests 62, 63, 64, and 65 (table 2) with tests 45, 46, 48, and 49 (table 1).

SUMMARY AND CONCLUSIONS

Some 50 salts generally present either in soils or in spray waters were tested in the laboratory to determine to what extent they can form soluble arsenic when in contact with acid lead arsenate. The salts were mixed in various concentrations with constant quantities of lead arsenate (about 3 pounds per 100 gallons) and allowed to stand for 24 hours with frequent shaking. The filtrates of these mixtures were analyzed for soluble arsenic.

Of the 10 different groups of salts tested, the nitrates, sulfates, and acetates, without a single exception, proved relatively nonreactive with PbHAsO_4 .

The chlorides, silicates, and bicarbonates produced moderate quantities of soluble arsenic.

Salts of carbonates and sulfides usually formed large percentages of soluble arsenic.

In the phosphate group, the three phosphates of calcium and the monobasic phosphates of sodium and potassium formed inappreciable quantities of soluble arsenic, whereas the dibasic and especially the tribasic phosphates of either sodium or potassium formed large amounts of soluble arsenic.

The percent of solubility of a salt in water is, by itself, not a determining factor in forming soluble arsenic. But, of a group of salts possessing the property of decomposing lead arsenate, the salt which is highly soluble in water will form more soluble arsenic than a salt which is only slightly soluble in water.

The hydrogen-ion concentration of the salt solution plays, with few exceptions, an important role in decomposing lead arsenate. Salts with pH values of 8 to 11.4 produced, with two exceptions, more soluble arsenic than did salts with lower pH values.

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FURTHER STUDIES ON GROWTH SUBSTANCES IN RELATION TO THE MECHANISM OF THE ACTION OF RADIATION ON PLANTS¹

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INTRODUCTION

It has been repeatedly demonstrated that ultraviolet and visible violet radiation have definite formative effects upon growing plants. One of the most striking of these effects is the general stunting of the plant. This was especially evident in the work of Schanz (12),² Popp (7), and Popp and Brown (8, 9, 10). In all this work no effect has been more consistently evident, except destruction by the extremely short wave lengths, than the decreased stature of the plants exposed to the blue-violet end of the spectrum.

In a previous study, Popp and McIlvaine (11), in an attempt to explain this formative effect of the short wave lengths, exposed seedlings to radiations from a mercury-vapor lamp in quartz, using the unscrubbed arc and three types of Corning glass filters: Noviol O, transmitting down to 389 m μ ; G586A, transmitting the region 300-436 m μ ; and red-purple Corex A, transmitting the region 250-415 m μ . These plants were tested for growth substance by a modified Went technique (3, 14), as were control plants kept in the dark. It was found that the wave length of radiation, the stature of the plants, and the amount of growth substance revealed by the tests, were all positively correlated. The shorter the wave length, the shorter were the plants and the smaller the amount of growth substance found. It seemed evident that if the Went technique is a reliable quantitative test for growth substance, then radiations, especially of the shorter wave lengths, have a destructive influence on the growth substance in the plant. This explains at least part of the mechanism of the action of radiation on plants. The present investigation was undertaken in continuation of the above study. In the work herein described more attention was given to the visible region of the spectrum. A study was also made of the effect on growth-substance content when seedlings were transferred from light to darkness and from darkness to light.

As early as 1933 there was presented some evidence indicating that light had some effect on growth substance. All this work, however, dealt with more mature plants. It was reported (1, 5, 6, 13, 15) that there is a higher concentration of growth substance in young illuminated plants than in those kept in the dark. Skoog and Thimann (13) concluded that light favors the formation of the growth substance in plants, and darkness favors its physiological activity. Later Avery, Burkholder, and Creighton (2), using fully matured

¹ Received for publication August 3, 1939. Contribution No. 125, Department of Botany, Pennsylvania State College. Paper No. 918 in the Journal Series of the Pennsylvania Agricultural Experiment Station. This work was supported by the radiation committee of the Division of Biology and Agriculture, National Research Council.

² Italic numbers in parentheses refer to Literature Cited, p. 215.

plants, found that the growth substance disappeared in darkness, but reappeared when the plant was replaced in the light. They concluded that light is necessary for the synthesis of the growth substance. They also reported some evidence that red light especially favors such synthesis.

Burkholder and Johnston (4) reported in a paper reviewed more fully elsewhere (11) that growth substance was destroyed by the shorter wave lengths of light, after such substance had been allowed to diffuse into agar blocks. They also found that such light inhibited the growth of seedlings, and by making correlated tests for the growth substance, produced some evidence that this inhibition was the result of the inactivation of the growth substance.

EXPERIMENTAL PROCEDURE

The plants used for the tests were turnips (*Brassica rapa* L.), of the Purple Top White Globe variety. Selected seeds were germinated on moist cotton and filter paper in sterile Petri dishes, 50 seeds to a dish. The seedlings were kept in darkness at 25° C. except during the radiation treatments. In some cases other conditions were maintained. These are noted below.

The sources of radiation were (1) daylight, (2) a 500-watt Mazda lamp in reflector, (3) a General Electric mercury-vapor lamp in glass, rated at 100 watts, and (4) a mercury-vapor lamp in quartz. The intensity of the last-mentioned lamp at 50 cm. with no filter was 21.65 ($10^{-5}/\text{cm.}^2$) watts. In table 1 are shown the types of Corning glass filters used, with their spectral ranges of transmission in the visible and ultraviolet regions and the comparative amount of energy they transmit, the transmission of the violet being unity. The total energy transmitted by each of these glasses was measured by means of a pyrheliometer of the Kimball and Hobbs type³ and either the distance from the lamp or the time of exposure, or both, were altered to equalize the energy through the filters. In some cases cheese-cloth screens were used to lower intensities. The time and distance of exposure were based upon exposure to the unscreened mercury-vapor arc, this latter being enough to provide a maximum of the formative effects in the plant with a minimum of injury.

TABLE 1.—*Spectral range (visible and ultraviolet regions) and energy transmission¹ of the filters used*

Filter	Spectral range	Comparative energy transmitted from—	
		Mercury-arc source	Mazda source
	mμ		
G34.....	529-720	5	20
G403ED.....	374-585	5	8
Correx D, clear.....	260-720	22	30
H R Signal Red.....	610-685	13	24
Sextant Green.....	465-600	4	5
Violet.....	380-482	1	1

¹ Violet as unity.

³ Lent by the Eppley Laboratory, Inc.

The plants were usually exposed to the particular radiation 24 hours after being placed in the germinators and semidaily or daily thereafter for $3\frac{1}{2}$ or 7 days. During the radiation a strong current of air was kept moving over the plants to minimize the heating effects of the lamp and to disperse the ozone when the latter was formed by the mercury-vapor lamp in quartz. Controls consisted of plants grown under the same conditions maintained for the test plants, except that irradiations were omitted.

Two groups of plants were kept in diffused daylight in the greenhouse with various periods of darkening, to determine the effect of darkness following growth in the light. In this case controls consisted both of plants grown in total darkness and plants grown continuously under greenhouse conditions.

Tests for the growth substance were made by a modified Went *Avena* curvature method (3, 14). The test coleoptiles were from oats (*Avena sativa* L.) of a uniform pure line, received from Dr. C. F. Noll, of the Pennsylvania Agricultural Experiment Station. In the growth-substance determinations about 1 mm. of the tip of each test seedling was cut off. These tips were then placed unilaterally on the ends of *Avena* coleoptiles which had been decapitated and placed in a dark moist chamber 2 hours previously. The coleoptiles bearing the test tips were placed in a moist chamber in the dark. Two hours later they were removed and the curvature of the coleoptile, if any, was measured in a shadow box. During all the manipulations the *Avena* seedlings were secured in special glass holders. All manipulations were carried out in photographically inactive light. That this light had no formative effect upon the *Avena* coleoptiles was determined by previous tests.

As a further check, growth substance was allowed to diffuse into agar blocks from the tips of turnip seedlings grown in the dark. These blocks were then placed in a moist chamber and irradiated by a mercury-vapor lamp in quartz through a screen of clear Corex D (2 mm.) glass. After this they were placed unilaterally on prepared, decapitated *Avena* coleoptiles which were then treated in the usual manner, described above. The control consisted of the same procedure, omitting only the irradiation of the blocks.

RESULTS

In table 2 are given the results of tests on seedlings grown for 7 days, receiving the radiations of a 500-watt Mazda lamp at 82 cm. for a total of 720 minutes every 24 hours. To minimize the heating effect, this radiation was administered 15 minutes at a time, alternating with 15-minute periods of darkness. Records of the mean heights of the seedlings were kept, and it will be noted that these were positively correlated with the amounts of growth substance indicated by the tests. These two filters, G34 and G403ED, divide the visible spectrum into two regions, there being only a small overlap in their regions of transmission. G34 transmits the red half of the spectrum and G403ED, the blue-violet half. Seedlings exposed to the shorter wave-lengths were uniformly shorter and contained less growth substance than those under the G34 glass.

TABLE 2.—Mean curvatures obtained from turnip test plants $3\frac{1}{2}$ and 7 days of age under various conditions of radiation when screens dividing the visible spectrum into 2 parts were used

PLANTS AGED 7 DAYS, AFTER RECEIVING RADIATIONS FROM A 500-WATT MAZDA LAMP A TOTAL OF 720 MINUTES EVERY 24 HOURS

Screen	Tests	Mean height of seedlings	Mean curvature
	Number	Centimeters	Degrees
G34.....	768	5.63	4.43±0.34
G403ED Blue.....	768	5.01	3.68±.31

PLANTS AGED $3\frac{1}{2}$ DAYS, AFTER RECEIVING RADIATIONS FROM A MERCURY-VAPOR LAMP IN QUARTZ FOR 5 MINUTES EVERY 12 HOURS

G34.....	720	3.50	4.09±0.02
G403ED Blue.....	720	2.60	3.00±.21

PLANTS AGED $3\frac{1}{2}$ DAYS, AFTER RECEIVING RADIATIONS FROM A 500-WATT MAZDA LAMP FOR A TOTAL OF 480 MINUTES EVERY 12 HOURS AND RADIATIONS FROM A MERCURY-VAPOR LAMP IN GLASS A TOTAL OF 60 MINUTES EVERY 24 HOURS

G34.....	732	3.75	4.28±0.30
G403ED Blue.....	780	2.51	3.09±.21
Control (total darkness).....	840	6.20	5.81±.26

In table 2 are also shown the results of irradiating the seedlings through the same screens but with a mercury-vapor lamp in quartz as the source of radiation. Exposures were for 5 minutes every 12 hours, and plants were grown for $3\frac{1}{2}$ days. In this case there was a greater difference between the two series of experiments than when the source was a Mazda lamp. The shorter plants were those under the blue glass, and these also contained less of the growth substance.

In table 2 are shown the results obtained from plants grown under radiation from both types of sources. The seedlings received the radiations from the Mazda lamp for a total of 480 minutes every 12 hours for $3\frac{1}{2}$ days. In addition, they received the radiation from a mercury-vapor lamp in glass at 15 cm. for a total of 60 minutes every 24 hours during the same period. The difference between the two groups was greater than in either of the preceding cases. The seedlings under the blue glass were here, also, shorter and contained less growth substance.

Several series of tests were made in which the visible spectrum was divided into three parts by the use of red (H R Signal Red), green (Sextant Green), and violet screens and comparisons made with the full spectrum (Corex D). In these series the total radiation reaching the plants was equalized on the basis of the total energy transmitted through the filters. This did not take into account differences of transmission in the infrared region, and since the total transmission of the Sextant Green screen and of the Violet screen were extremely low as compared with the H R Signal Red screen, results obtained with these screens are only partly to be attributed to the wavelength region in the visible spectrum transmitted by each of these screens. In fact, from previous results reported, it seems likely that even when the total intensities were equalized under the three screens the plants under the green and the violet screens received less visible radiation than did those under the red screen.

TABLE 3.—Mean curvatures obtained from turnip test plants 7 days of age under various conditions of radiation when screens dividing the visible spectrum into 3 parts were used

PLANTS THAT RECEIVED RADIATION FROM A 500-WATT MAZDA LAMP FOR A TOTAL OF 720 MINUTES EVERY 24 HOURS

Screen	Tests	Mean height of seedlings	Mean curvature
	Number	Centimeters	Degrees
Corex D (2 mm.)	576	1.60	1.27±0.027
H R Signal Red	576	2.97	3.48±.39
Sextant Green	576	4.89	5.64±.32
Violet	576	4.55	5.00±.17

PLANTS THAT RECEIVED RADIATION FROM A 500 WATT MAZDA LAMP FOR A TOTAL OF 720 MINUTES EVERY 24 HOURS AND FROM A MERCURY-VAPOR LAMP IN QUARTZ A TOTAL OF 15 MINUTES EVERY 24 HOURS

H R Signal Red	480	3.02	3.21±0.25
Sextant Green	480	4.95	5.43±.29
Violet	480	1.96	2.12±.22
Control (total darkness)	702	8.30	5.90±.17

PLANTS EXPOSED TO DAYLIGHT THROUGH PLATE GLASS AND WATER AND OTHER SCREENS

Corex D (2 mm.)	288	1.49	1.92±0.27
H R Signal Red	288	3.50	3.92±.37
Sextant Green	288	5.21	5.56±.33
Violet	288	5.00	4.96±.28

The results obtained with these screens are presented in table 3, which gives first the results obtained when a Mazda lamp was used as the source of radiation. The plants were irradiated for a total of 720 minutes every 24 hours. The radiation was in periods of 15 minutes alternating with 15-minute periods of darkness. The shortest plants were those under the clear Corex D screen, and these plants also contained the smallest amount of growth substance. Next in height and amount of growth were the seedlings under the H R Signal Red filter. The plants under the Violet screen were still taller and contained more growth substance, while those under the Sextant Green glass were the tallest and contained the most growth substance.

It is interesting to note that the plants under the Sextant Green screen as well as those under the Violet screen were taller and gave higher curvatures in the *Arena* test than did those under the red screen in spite of the fact that those under the red screen received only longer wavelengths of radiation. This may be attributed to the factors mentioned in a previous paragraph as well as to the fact that the Mazda lamp is relatively deficient in blue-violet radiation. That this is true is indicated in table 3, where the radiation from the Mazda lamp was supplemented by that from a mercury-vapor lamp which is rich in blue-violet rays. In this case, the plants under the violet screen were the shortest and contained the smallest amount of growth substance. Plants under the red glass were next in both respects, and those under the Sextant Green screen were tallest and contained the most growth substance. Controls in complete darkness exceeded the test plants in every case. The higher intensity of the blue-violet region under these conditions did not offset the effects under the Sextant Green screen

since this screen does not transmit much of the extreme blue-violet region.

Table 3 contains the results of growing plants in sunlight for one-half day and in diffused daylight one-half day every 24 hours for 7 days, through August. Four different screens were used over separate plant cultures, but, in addition, over all the plants was a plate-glass screen covered with a flowing film of cold water to minimize the heating effects of the sunlight. Under these conditions seedlings under the clear Correx D screen were the shortest and contained the smallest amount of growth substance. Next in height and amount of growth substance were those under the red glass. Seedlings under the violet screen came next in height and amount of growth substance, and the tallest plants, with the greatest amount of growth substance, were those under the green filter. Since intensities were not accurately equalized in this group, the greater height of the plants under the green and violet screens may be attributed to the low amount of radiation transmitted by them.

Table 4 contains the results of irradiating agar blocks into which had diffused growth substance from the tips of turnip seedlings grown in total darkness, the exposure being one 15-minute period at 50 cm. The amount of active growth substance in such blocks was not appreciable. The control blocks produced the usual amount of active growth substance. The *Avena* test coleoptiles were used at the age of $3\frac{1}{2}$ days, during all of which time they had been growing in the dark.

Two series of experiments were carried out to determine the effect of various periods of light and darkness upon seedlings. In the case of the seedlings itemized in table 5, all tests were made at the end of 7 days. There was some evidence of accumulation of growth substance, but the amount was evidently small. The smallest amount was found in seedlings grown in diffused daylight for 7 days. The control had the largest amount and the other two groups were between these. Those in the light the last 3 days had less growth substance than those in the dark the last 3 days.

TABLE 4.—Mean curvatures obtained from *Avena* coleoptiles $3\frac{1}{2}$ days of age after irradiating the test blocks ¹ with a mercury-vapor lamp in quartz

Item	Tests	Mean curvature
	Number	Degrees
Irradiated agar blocks	480	0.02±0.00
Control	492	5.84±0.17

¹ Containing diffused growth substance from tips of turnip seedlings grown in total darkness.

TABLE 5.—Mean curvatures obtained from turnip test plants 7 days of age after exposure to various conditions of diffused daylight and darkness

Conditions	Tests	Mean height of seedlings	Mean curvature
	Number	Centimeters	Degrees
Control (total darkness)	588	5.40	5.08±0.42
In dark 4 days, then in light 3 days	600	3.75	3.32±.35
In light 4 days, then in dark 3 days	576	4.58	4.80±.32
In diffused daylight 7 days	588	3.00	2.39±.33

In table 6 are shown the results of various periods of diffused daylight and darkness in three series of experiments. These seedlings were grown in soil in the greenhouse, and the days are reckoned from the time the plants appeared above the soil. Each series can be considered as a single culture. For instance, in series A, about 700 seeds were planted. These had been above the ground for 3 days when 132 of them were tested for the growth substance. The rest were placed in darkness for 3 days, at the end of which time another sample (132) of them was tested. The remaining seedlings were placed in diffused daylight and at the end of 3 days more these were tested. Controls were grown for the 9 days in either complete darkness or in diffused daylight, with no changes. There was little accumulation of the growth substance evident from the results obtained. Seedlings that had been in the dark always contained more of the growth substance than did those that had been in the light, regardless of conditions existing previously.

TABLE 6.—Mean curvature obtained from turnip seedlings transferred from light to darkness and from darkness to light

Series and conditions	Tests	Mean curvature
Series A:	Number	Degrees
After 3 days of diffused daylight	132	2.46±0.20
After 3 days of darkness following the 3 days in daylight	132	4.90±.14
After 3 more days of diffused daylight following the 3 days in darkness	132	2.61±.17
Control—9 days of diffused daylight	144	2.55±.23
Control—9 days of darkness	132	5.03±.27
Series B:		
After 2 days of diffused daylight	144	2.53±.18
After 4 days of darkness following the 2 days in daylight	144	4.96±.17
After 2 more days of diffused daylight following the 4 days in darkness	144	2.89±.21
Control—8 days of diffused daylight	144	2.48±.18
Control—8 days of darkness	144	5.11±.26
Series C:		
After 4 days of diffused daylight	132	2.40±.19
After 4 days of darkness following the 4 days in daylight	132	4.79±.16
After 3 more days of diffused daylight following the 4 days in darkness	132	2.72±.21
Control—11 days of diffused daylight	132	2.45±.16
Control—11 days of darkness	132	4.99±.24

DISCUSSION

Throughout the experiments there was found to be a very positive correlation between the heights of the seedlings and the amounts of growth substance present in them as indicated by the *Arena* curvature technique. It was also found that under equal amounts of energy the heights of the plants were less when the seedlings were exposed to shorter wave lengths of radiation. Similarly, the shorter the wave lengths of radiation, the smaller was the amount of growth substance present. When the visible spectrum was divided into three sections by the use of red, green, and violet screens, the plants under the green screen were the tallest and contained the most growth substance. This probably may be attributed to the fact that the radiation passing through these screens was not equalized in the visible region of the spectrum but on the basis of total energy transmitted, including infrared. Furthermore, the relative deficiency of blue-violet radiation in the Mazda lamp source caused plants under the violet screen to be taller than those under the red screen (table 3). The greater height of the plants under the green screen and the greater amount of growth

substance they contained might be thought to indicate that green light has less inactivating effect on growth substance than does red light, but this cannot be proved until plants have been grown under both kinds of light under accurately equalized energy.

When the visible spectrum was roughly divided into two parts, by the use of the G34 and G403ED screens, and intensities equalized, the seedlings under the blue glass were always shorter and contained less of the growth substance than did those under the yellow glass. The source of radiation caused some of the differences between the two. It is evident from the data that the blue end of the spectrum has an inhibiting effect on elongation and a correlated inactivating effect on growth substance. When the source was a Mazda lamp, the difference between the two groups was significant, although not great. When sources richer in the blue and violet radiations were used, the amount of growth substance and the height of the seedlings were considerably less than in the plants under the G34 screen. It is believed safe to assume that the lesser height of the plants under the blue screen is caused by a greater inactivation of the growth substance by the shorter wave lengths of radiation. It is also believed that the red light has some inactivating effect, although not nearly so much as blue-violet light.

In the tests in which seedlings were transferred from light to darkness and from darkness to light, the plants always contained less growth substance after being in the light and more after being in the dark. Although several periods of light and darkness were used, results were always the same. Obviously in seedlings of this age, light causes inactivation of the growth substance and darkness favors its production or activity.

SUMMARY

This study was carried out in continuation of an earlier one by Popp and McIlvaine. The previous work had indicated that the short wave-length radiation, from 250–415 $m\mu$, inhibited the growth of seedlings by inactivating the growth substance in the shoot tips. The present work enlarges the study by showing the effect of radiation of wave lengths 374–720 $m\mu$.

A number of turnip seedlings were grown and subjected to visible radiation and to various conditions of daylight and darkness. Heights of the plants were recorded, and the amount of growth substance present after the treatments was determined by a modified Went *Avena* curvature technique. The effect of the shorter wave-length radiation on growth substance in agar was similarly determined.

It was found that when the visible spectrum was divided in half, under approximately equalized energy, plants grown under the blue-violet half were shorter and contained less growth substance than those under the red half.

When the spectrum was divided into three parts (red, green, and blue violet), it was not possible under the conditions used in these experiments to accurately equalize energy in the visible region. Consequently, the plants under the green region were the tallest, probably having received the least light. Whenever a source rich in blue-violet rays was used, the plants under the blue violet were always the shortest. In all cases, the tallest plants had the most growth substance and the shortest the least.

Growth substance allowed to diffuse from turnip-stem tips into agar was inactivated by the radiation from a mercury-vapor lamp.

When seedlings were transferred from light to darkness and from darkness to light, they always contained less growth substance after being in the light and more after being in the dark.

In general, these results substantiate the conclusions of the authors in their previous paper that the formative effect of radiation may be at least partly explained on the basis of the effect of radiation on growth substances in the plant.

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JOURNAL OF AGRICULTURAL RESEARCH

VOL. 60

WASHINGTON, D. C., FEBRUARY 15, 1940

No. 4

BACTERIAL WILT RESISTANCE AND GENETIC HOST-PARASITE INTERACTIONS IN MAIZE¹

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INTRODUCTION

Bacterial variation has been studied mainly in vitro, few investigations having been made on the effect of host passage on the pathogen. The common belief is that virulence is maintained or enhanced by passage through a host. Outstanding as a critical analysis of this problem is the work of Wellhausen (19),³ who determined the effect of repeated passage of *Phytophthora stewartii* (E. F. S.) Bergey et al.⁴ through resistant and susceptible lines of maize and through certain other grasses. Successive passage through susceptible maize decreased virulence, while successive passage through resistant maize increased virulence.

This investigation has been devised to check and extend Wellhausen's observations on the effect of the host upon bacterial virulence and to determine the factors that operate to bring about such changes. The same organism, *Phytophthora stewartii*, has been used.

REVIEW OF LITERATURE

Smith (14), the first to study *Phytophthora stewartii*, considered it to be very stable in culture. In 1918 McCulloch (10) reported two distinct types of colonies, both remaining stable for at least 2 years. Other workers (5, 6, 22) report that atypical strains of *Ph. stewartii* may be isolated from infected soil or plants. Recently McNew (11) isolated single colonies from dilution plates of virulent cultures of *Ph. stewartii* and found stock cultures to be composed of many variants as regards virulence. Later, working with 10 strains of *Ph. stewartii* differing in virulence, McNew (13) found the strains also differed in other physiological reactions. Ability to use inorganic nitrogen was correlated positively with virulence. From studies of 22 cultures Ivanoff et al. (8) report that *Ph. stewartii* includes several types of organisms separable into 3 primary types on the basis of morphological, physiological, and colony characteristics. Positive correlation between amount of gum and pathogenicity was indicated. A similar correlation between consistency of growth on agar and virulence also was observed by Wellhausen (19). The cultural and physiological similarities between cultures of *Ph. stewartii* and *Ph. vasculorum* have been noted by Ivanoff (7).

¹ Received for publication June 6, 1939. Journal paper No. G-955 of the Iowa Agricultural Experiment Station, Genetics Section, project 404. Taken from a thesis submitted to the faculty of the Graduate College, Iowa State College, in partial fulfillment of the requirements for the degree of doctor of philosophy. The complete thesis is on file in the Iowa State College Library.

² The author wishes to express his appreciation to Dr. E. W. Lindstrom and Dr. J. W. Gowen for suggestions and criticism throughout the progress of this work.

³ Italic numbers in parentheses refer to Literature Cited, p. 238.

⁴ The nomenclature of this species is debatable and is here used to designate the nonmotile bacterium of Stewart that is the causal organism of bacterial wilt of maize. It is also known as *Bacterium stewartii* E. F. S. and as *Aplanobacter stewartii* (E. F. S.) McCulloch.

Allen and Baldwin (1) determined the effect of repeated plant passage of effective and ineffective strains of *Rhizobium*, finding that effective strains may become ineffective, or ineffective strains may become effective during repeated plant passage.

Hendrickson et al. (4) passed the crown gall bacterium through the tomato, finding variation in the physiological behavior of the crown gall organism during plant passage, but noting no effect upon virulence.

Webster and Clow (17) found that strains of pneumococcus vary widely in virulence. Serial passage from nose to nose consistently failed to raise the internasal virulence of initially low virulent strains; serial internasal passage of strains with high initial virulence reduced internasal virulence almost to zero without inducing any change in the interperitoneal virulence. Earlier, Webster (16) had observed that virulent strains of *Bacterium lepioteticum* may be replaced in the nasal passages of rabbit by relatively avirulent strains.

With mouse *Pasteurella*, Greenwood et al. (2) found avirulent strains arising by passage through immunized animals, whereas strains obtained from nonimmunized mice were as virulent as the original strain. In one instance, a virulent epidemic of mouse typhoid arose by emergence of a virulent variant from a relatively avirulent strain of *Bacillus aertrycke*.

The criticism of most investigations on host passage is that host resistance has not been kept a constant, hence the cause of any bacterial variation is difficult to interpret.

EXPERIMENTAL MATERIALS AND METHODS

Host material for these virulence studies was limited largely to four lines of maize (*Zea mays* L.) inbred for at least 10 generations. A yellow dent line, OSF, was the resistant host, while a white flint line, WF, and two yellow sweet lines, GB134 and GB797, were used as highly susceptible hosts. GB134 was used in passage work, while GB797 was used for all virulence tests.

Resistant line OSF when inoculated with virulent *Phytomonas stewartii* showed characteristic lesions only on those leaves that were actually expanded at the time of inoculation, subsequent leaves being free from lesions. Stunting of the plant was slight. Susceptible lines GB134 and WF died soon after inoculation, usually within 2 weeks. With these lines the initial symptoms were the characteristic wilt lesions. The bacteria soon became systemic throughout the vascular system, a condition which resulted in a wilting and was soon followed by drying and death. GB797 reacts like GB134 or WF except that it is slightly more resistant and dies somewhat more slowly. Wellhausen (20) determined the genetic basis of resistance between these lines as being primarily influenced by three supplementary dominant factors.

Several cultures⁵ of *Phytomonas stewartii* were tested for virulence, and from this group 6 cultures that represented a range of virulence from high to low were selected for further use. As a check on their purity all cultures were plated, observed, and a subculture of each stock made of a composite of 15 typical colonies from that stock. After this purification the stocks were continued on nutrient dex-

⁵ These cultures were obtained through the courtesy of Drs. E. J. Wellhausen, S. S. Ivanoff, C. B. Elliott, and J. H. Muncie.

trose agar. Cultures were transferred once every 4 weeks, allowed to grow at room temperature for 24 hours, and then stored in the refrigerator at about 5° C. Strain characteristics are summarized in table 1. On nutrient dextrose agar the more pathogenic strains form larger, more viscid or mucoid colonies than do the less pathogenic strains.

TABLE 1.—*Growth characters on nutrient dextrose agar of bacterial stocks used for host passage*

Culture No.	Virulence index ¹	Characteristics of 48-hour agar plate colonies				Generation time
		Diameter ²	Consistency	Surface	Color	
		<i>Microns</i>				<i>Hours</i>
S15.....	36	1,880±120	Dry, granular	Rough	Orange yellow	2.3
D3.....	45	2,780±520	do	do	Lemon yellow	2.9
A87.....	48	6,080±1,740	Butyrous	Smooth	do	2.0
FB32.....	60	4,400±880	do	do	Creamy yellow	2.2
A14.....	77	11,000±1,520	Mucoid	do	do	1.8
O23.....	72	6,040±1,460	Butyrous-viscid	do	do	3.2

¹ Virulence index = $100 - \frac{\text{Green weight of test plants} \times 100}{\text{Green weight of check plants}}$

² Standard deviation calculated on 300 colony measurements.

Bacteria for inoculum were grown on agar slants for 24 hours, washed off into 5 cc. of nutrient broth, and without further incubation inoculated into test plants. Host plants were inoculated by injecting a small quantity of inoculum into the first nodal region of the plant with a hypodermic syringe. The actual amount of inoculum for each plant consists of the liquid remaining in the needle hole after the syringe needle is withdrawn. The percentage of infection was greater than 99 percent. Reisolations were made 14 to 16 days after inoculation, 8 to 10 plants from each treatment being used. For colony observations, surface colonies were obtained by means of a poured-plate, smear technique.

After reisolation the new passage culture was made up of a large number of colonies (from 50 to 100) picked at random, pooled into one culture, and treated like the stock cultures.

In passage experiments, virulence was determined after two, four, six, and eight passages on a susceptible inbred, GB797. The test plants were grown in greenhouse benches. Not less than 15 plants were inoculated with each culture. Each test culture was replicated three times and compared in virulence with the initial stock culture. After the eighth passage all cultures were given comparative tests against the stock culture and against each passage stock. An analysis of variance (15) was made to test the significance of virulence trends observed during host passage.

Green weights of each block of plants were taken 14 days after inoculation. A virulence index was calculated as

$$100 - \frac{\text{Green weight of test plants} \times 100}{\text{Green weight of check plants}}$$

This index may vary from zero to 100, the index increasing as virulence of the bacteria increases. Green weight means the weight of

the top above the first node. Another virulence index has been used and described by McNew (11) and Wellhausen (19).

The procedure in designating passage cultures has been to list the stock culture, the maize line through which it passed, and the number of passages. Thus S15-GB-8 means the culture obtained by passage of stock culture S15 through maize line GB134 for eight successive passages.

EXPERIMENTAL RESULTS

EFFECT OF SUCCESSIVE HOST PASSAGE ON THE VIRULENCE OF SIX STOCK CULTURES

Subcultures of six stock cultures were passed successively through one resistant and two susceptible lines of maize. Tests for virulence were made after two, four, six, and eight passages. A virulence comparison with the original strain was made at each test, and after eight passages all passage cultures were compared with each other and with the stock strain. The virulence of the stock culture and of the passage strains is given in table 2. There is a pronounced trend for decrease of virulence after passage through the susceptible lines GB134 and WF, and for increase by passage through the resistant line OSF. To such a generalization there is only one minor exception—that of the FB32-WF passage.

TABLE 2.—Virulence indices of stock and passage cultures during eight successive passages through susceptible and resistant hosts

Host line	Host passages	Virulence indices of strain ¹ —					
		S15	D3	A87	FB32	O23	A14
GB134 (susceptible).....	<i>Number</i>						
	0	36	45	48	60	72	77
	2	27	32	37	48	52	54
	4	19	16	31	50	50	45
	6	21	12	17	33	41	36
	8	18	10	17	27	19	29
Difference ²		-18	-35	-31	-33	-53	-48
WF (susceptible).....	0	36	45	48	60	72	77
	2	39	41	43	64	70	62
	4	22	46	37	71	47	54
	6	25	53	41	68	35	36
	8	19	33	33	65	28	28
Difference ⁴		-17	-12	-15	+5	-44	-49
OSF (resistant).....	0	36	45	48	60	72	77
	2	45	43	72	54	78	71
	4	58	52	68	63	68	72
	6	60	61	70	72	70	71
	8	56	63	67	65	74	73
Difference ⁵		+20	+18	+19	+5	+2	-4

¹ See footnote 1, table 1, and text p. 219.

² This is a second series. In the first series the passage culture became completely avirulent apparently because of the culturing of a contaminant.

³ Differences of less than 20 are not statistically significant.

⁴ Differences of less than 23 are not statistically significant.

⁵ Differences of less than 18 are not statistically significant.

The S15 and O23 passage series are shown in figures 1 and 2 respectively, where the change in virulence during passage is a progressive trend beginning immediately on passage. In the S15 passage, the ultimate reduction in virulence is brought about within the first four

passages through the susceptible line. Through the resistant line, increase in virulence was most rapid in the early passages but apparently was still rising when the experiment was stopped.



FIGURE 1.—Comparative virulence of S15 stock and passage strains of *Phytomonas stewartii* on a susceptible line of maize 15 days after inoculation: A, Successive passage of S15 stock through susceptible host GB134; B, successive passage of S15 stock through resistant host OSF. a, Check; b, inoculated with stock S15; c, d, e, and f, respectively, inoculated with S15 passage strains after two, four, six, and eight successive host passages.

Changes in virulence during passage were associated with changes in type of colony growth on nutrient dextrose-agar plates. Loss in

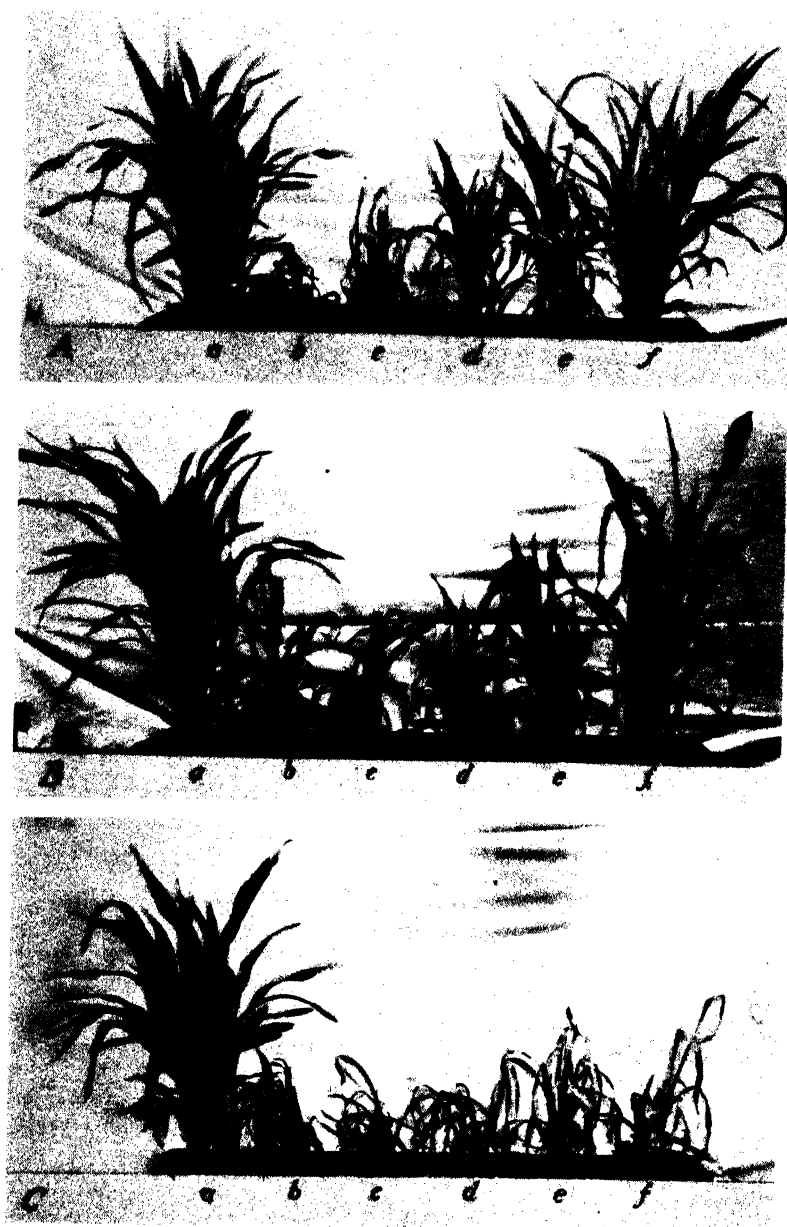


FIGURE 2.—Comparative virulence of O23 stock and passage strains of *Phytomonas stewartii* on a susceptible line of maize 15 days after inoculation: A, Successive passage of O23 stock through susceptible host GB134; B, successive passage of O23 stock through susceptible host WF; C, successive passage of O23 stock through resistant host OSF. a, Check; b, inoculated with stock O23; c, d, e, and f, respectively, inoculated with O23 passage strains after two, four, six, and eight successive host passages.

virulence is associated with a raised, firm colony. Of the four initially smooth cultures passed through the susceptible host—A87, FB32, O23, and A14—in only A14 was the change from smooth to rough phase associated with the decrease in virulence; in the others the colonies remained in the smooth phase, becoming drier, firmer, and more intensely colored. The initially rough S15 and D3 cultures decreased in virulence during susceptible host passage, and remained in the rough phase without apparent colony type changes.

Associated with an increase in virulence by passage through the resistant host, was a more spreading, watery, and viscid growth of the colonies on agar. The change from rough to smooth was observed in passage cultures of both the initially rough stocks, and the increase in the proportion of smooth type was associated with the increase in culture virulence. The highly virulent A14 and O23 cultures remained constant on resistant host passage; A87 and FB32 growth became more watery and spreading as virulence increased.

CHANGE OF VIRULENCE IN PASSAGE STOCKS BY REVERSING HOSTS

To determine whether virulence of a passage culture could be again changed by reversing the host through which it passed, virulent strains that had been passed through resistant OSF for eight times were successively passed through susceptible GB134, and sister avirulent cultures obtained after eight passages through GB134 were passed through OSF. Six successive reverse passages through the new host were made, and virulence determined after two, four, and six passages (table 3).

TABLE 3.—*Change in virulence of passage cultures after the host through which the bacterial stocks were passed had been reversed*

Host line	Host passages	Virulence indices of strain ¹ -			
		S15-GB-8	A87-GB-8	O23-GB-8	A14-WF-8
OSF (resistant)	<i>Number</i>				
	0	18	17	19	28
	2	24	36	57	40
	4	41	56	64	47
	6	51	66	70	59
Difference ²		-33	-49	-51	-31
Host line	Host passages	S15-OSF-8	A87-OSF-8	O23-OSF-8	A14-OSF-8
	<i>Number</i>				
GB134 (susceptible)	0	56	67	74	73
	2	43	52	50	65
	4	36	42	37	44
	6	28	35	32	35
Difference ³		+28	+32	+42	+38

¹ See footnote 1, table 1, and text p. 219.

² Differences of less than 16 are not statistically significant.

³ Differences of less than 13 are not statistically significant.

During these reverse passages, changes in colony type and virulence were similar to those described earlier. Virulence of passage stocks

changed as readily and as rapidly as stock cultures that had no host passage.

VIRULENCE VARIATION WITHIN A STOCK CULTURE AND THE EFFECT OF
SUCCESSIVE HOST PASSAGE ON VIRULENCE VARIABILITY

As a further test of what changes take place within a bacterial population, a stock culture of intermediate virulence (index 48) was selected to test the virulence variability initially present in the culture as compared with that present after eight passages through susceptible and resistant hosts. Culture A88, selected for this purpose, had been held on agar slants for 3 years with no plating or host passage. One hundred single-cell isolates were obtained by following the plating method described by McNew (12). Each colony was plated successively three times, then put on agar slants and held as a stock culture. Virulence tests were made in triplicate.

It is recognized that the use of "single-cell" cultures is commonly limited to cultures derived by the hanging-drop method. McNew (12) by direct microscopic examination of poured plates of this species has shown that over 99 percent of the loci occupied by bacteria have only one cell, and after about 11 hours from 80 to 94 percent of these cells have divided, the number depending on the nutrient composition of the agar. Lincoln and Gowen⁶ has shown that 4 different strains of *Phytomonas stewartii* are inactivated by X-rays at the same general rate and a rate typical of single particles. As regards the question of whether a colony originates from 2 genetically different strains, Gowen and Lincoln⁷ by plating colonies from mixed cultures of white and yellow strains of *Ph. stewartii* found that only 4 colonies out of 2,072 had originated jointly from each of the two strains in the culture. It is further to be stressed, as McNew pointed out, that by making several serial platings and single-colony isolations the probability of obtaining a culture derived from a single cell at least once during this process is tremendously high. The evidence for the plate method of obtaining single-cell colonies or cultures with the species *Ph. stewartii* should be recognized. It is on the basis of this probability and evidence that the term "single cell" is used.

The 100 single-cell cultures from stock A88 varied widely in virulence, most of the colonies tending to possess the same general virulence as that of the parent stock (fig. 3, *a*). Mean virulence of the 100 isolates was 47 with a standard deviation of ± 21 . With respect to virulence this stock culture is a population composed of many variants. When such a culture is inoculated into maize, the host is subjected to invasion by a great number of bacterial variants. If certain variants should be better adapted than others to the microenvironment of the host, selective growth would occur, changing the composition of the population. Such has actually been found to be the case, for after 8 passages through the susceptible line the virulence index of the passage culture changed from 48 to 17, with a mean virulence index of 31 ± 16 in the 100 single-cell isolates (fig. 3, *b*). Passage through the susceptible

⁶ LINCOLN, R. E. and GOWEN, J. W. INACTIVATION RATE OF BACTERIA BY X-RADIATION IN RELATION TO WAVE LENGTH, TEMPERATURE, AND CELL SIZE. Manuscript.

⁷ GOWEN, J. W., and LINCOLN, R. E. A TEST FOR SEXUAL FUSION IN BACTERIA. Manuscript in Genet. Dept., Iowa State College.

host effected a definite shift in the bacterial population toward the avirulent type of organism.

With passage through the resistant line the virulence index of the passage culture changed from 48 to 67, with a mean virulence index of 64 ± 15 in the 100 single-cell isolates (fig. 3, c).

Obviously the change in virulence of the passage cultures is due to a shift in the ratio of virulent to less virulent forms initially present. This is contrary to concepts held by certain bacteriological pathologists. For example, Wilson (21, p. 310) states: "When a given strain

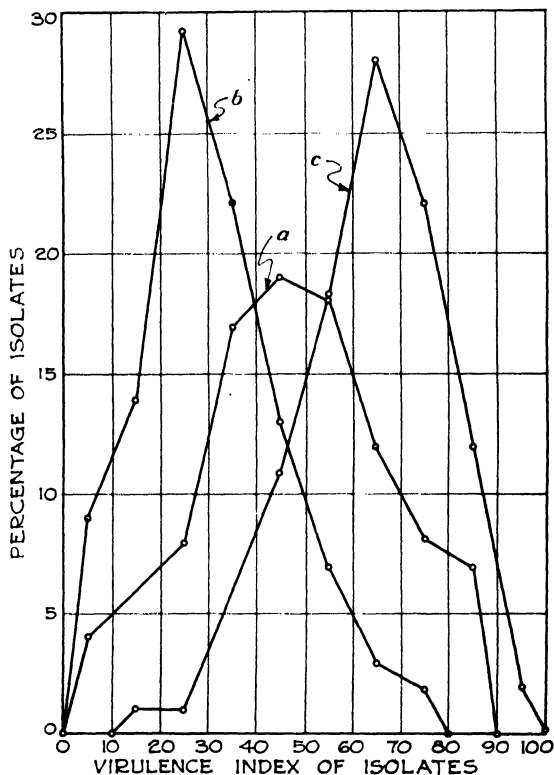


FIGURE 3.—Frequency distribution of pathogenicity of single-cell isolates of *Phytophthora stewartii* from a culture before and after host passage: a, Distribution of 100 isolates from initial stock culture; b, distribution of 100 isolates from passage stocks after 8 passages through susceptible host; c, distribution of 100 isolates from passage stock after 8 passages through resistant host.

comprises organisms showing discontinuous variation in virulence, the virulence of the whole culture is similar to that of the most virulent variants."

To test this hypothesis, inoculating mixtures were prepared that contained different proportions of virulent and avirulent bacteria. The virulence index of these mixtures was tested on susceptible maize and compared with the index of the pure strains. As shown in figure 4, virulence bears a direct relationship to the ratio of virulent to avirulent bacteria. Accordingly, the proportion of virulent and avirulent

types in the inoculum determines the virulence of that culture and not the virulence of the most virulent variant. There is no indication of an "all-or-none" reaction at any dilution in this organism.

HOST PASSAGE OF SINGLE-CELL ISOLATES

Progeny from single-cell stocks should be uniform for all characters if it be assumed that bacteria multiply only by simple fission. In such a culture selection for degree of virulence should be ineffective unless

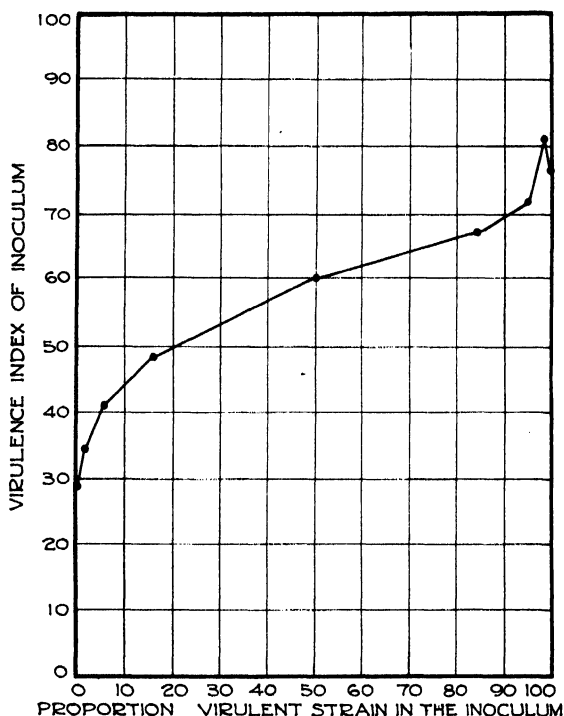


FIGURE 4.--Change in virulence index of inoculum composed of various proportions of virulent and avirulent bacteria.

variation arose. A critical test of whether virulence change is due entirely to selection of existing variation or whether adaptive variation can arise by some other means is furnished by host passage of single-cell cultures. Such a test was made by repeatedly passing virulent single-cell cultures through both resistant and susceptible maize lines and determining the effect of host passage on virulence and colony type.

Single-cell strains from the virulent A14 stock were passed through a susceptible host, but not all cultures were passed through the resistant host since previous work had indicated the lack of any noticeable change during passage of virulent bacteria through a resistant host by the technique of host passage described earlier. Passage was made for six successive times, virulence tests being made after two, four, and six passages (table 4).

TABLE 4.—Virulence indices and observed variation of single-cell cultures from A 14 stock after six successive host passages

Culture and stock No.	Initial viru- lence index ¹	Virulence indices after host passage								Colony variants	
		Susceptible host				Resistant host					
		Passage No.			Differ- ence ²	Passage No.			Differ- ence ²	Passage first observed	Virulence indices
		2	4	6		2	4	6			
Stock culture A14.....	77	66	44	33	-44	75	79	80	+3	2	29
Single-cell cultures:											
502.....	80	78	51	42	-38					3	13
504.....	83	81	74	63	-20	79	80	77	-6	3	43
507.....	73	60	31	56	-17					1	45
508.....	84	69	66	62	-22	75	76	80	-4	3	48
510.....	84	80	77	63	-21					5	50
511.....	82	73	65	54	-28					5	24
512.....	78	54	57	49	-29	77	73	79	+1	4	42
513.....	81	83	75	68	-13					3	54
516.....	85	58	41	24	-61	79	82	74	-11	2	35
517.....	70	67	57	53	-17					5	45
519.....	71	68	63	66	-5					None	
520.....	82	79	65	50	-32	77	75	80	-2	4	39
522.....	83	74	40	36	-47					2	30

¹ See footnote 1, table 1, and text p. 219.² Differences of less than 15 are not statistically significant.

Perhaps the most striking thing observed with these single-cell culture passages is the consistency with which all cultures that passed through the susceptible line decreased in degree of virulence, while those that passed through the resistant line retained their virulence without appreciable change. Notes on the particular passage in which atypical colony types were first observed are believed to be significant in showing that the atypical colony type was not present at the beginning of the passage, and its origin, from whatever source, appears to be a process occurring rapidly in some cultures, more slowly in others. In only one passage culture were atypical (rough mucoid) colony types observed immediately after the first passage, and in only one culture were atypical colonies not observed during the six passages.

During passage single-cell stocks of A14 shift in virulence and colony type in a manner comparable to the change of the original A14 stock culture. This observation somewhat parallels Hadley's (3) general statement on bacterial dissociation. He notes that the more recent work on bacterial dissociation, starting with single-cell cultures, is in no way different from earlier results that involved only colony isolations or in some cases apparently only mass inoculations.

DIFFERENTIAL HOST SELECTION OF VIRULENT AND AVIRULENT BACTERIA

Since certain virulent strains of *Phytophthora stewartii* are characterized by one type of colony and avirulent strains by an entirely distinct type of colony, in a mixture of these two, a morphological character may be used to identify the physiological potentiality (virulence) of a given bacterium. In previous experiments the fact that host constitution plays a definite part in the degree of virulence ultimately attained in the bacterial population has been demonstrated. An experiment was designed to furnish quantitative data on the rate at which change in a bacterial population takes place during host

TABLE 6.—*Change in proportion of virulent type colonies after passage of mixed cultures through the resistant and susceptible host, with average daily rate of change*

Date	Duration of passage	Initial proportion of virulent type colonies	Susceptible host GB134		Resistant host OSF	
			Proportion of virulent type colonies	Average daily rate of change (slope) ¹	Proportion of virulent type colonies	Average daily rate of change (slope) ¹
1938	Days	Percent	Percent		Percent	
Mar. 15.....	14	65	40	-1.96	72	+0.55
Do.....	14	85	68	-1.33	88	+ .22
Apr. 1.....	19	41	28	- .72	54	+ .72
May 8.....	14	80	63	-1.30	86	+ .49
Do.....	14	28	21	- .52	38	+ .73
May 22.....	20	65	49	-1.45	79	+ .74
Aug. 5.....	15	15	8	- .50	37	+1.49

¹ Figures in this column represent m in the formula for a straight line between 2 points, $m = \frac{y_1 - y_0}{x_1 - x_0}$

Strains of A14 and S15 were used for most of the mixed culture work. These were particularly favorable because they represented extremes

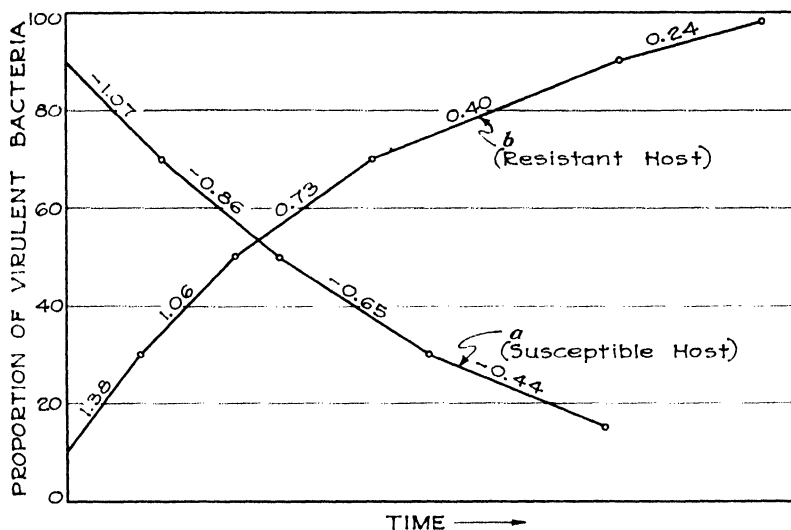


FIGURE 5.—Effect of host passage on proportion of virulent to avirulent bacteria in mixed culture inoculations: *a*, Susceptible host; *b*, resistant host. Figures denote regression coefficients in short experiments.

of virulence, were very stable in culture, and were distinct in colony morphology.

There is a highly significant positive correlation of $r=0.95$ between the ratio of virulent and avirulent bacteria in the inoculating suspension and that obtained by isolating from the first definite lesion on the leaf, showing that bacteria that actually grow and produce a diseased condition are reliably estimated by plating directly from the inoculating medium.

As shown earlier (9), upon passage of the mixed cultures through the susceptible host there is a differential selection favoring the avirulent type of bacteria. Figure 5, *a*, shows this change in an inoculum with

an initial proportion of 90 virulent to 10 avirulent bacteria decreasing during passage through the susceptible host to 15 virulent and 85 avirulent bacteria. The whole sweep of this reaction is curvilinear, apparently asymptotic to the replacement of the virulent type by the avirulent. Because of the early death of the susceptible host the full reaction is not expressed in any one experiment, but this curve is built up from the short experiments given in tables 5 and 6, in which the initial proportion of virulent to avirulent bacteria varied between 15 and 98 percent of the virulent type.

When passage is made through the resistant host there is a differential selection for the virulent type. Figure 5, *b*, shows the increase in the proportion of virulent bacteria during passage through the resistant host. Curves *a* and *b* were determined in a comparable manner, differing only in direction of change.

To test the conclusion that rate and direction of selection are dependent upon host resistance, the same technique and bacterial stocks were used in experiments in which 14 new maize lines with different degrees of resistance were employed as host. Host resistance as rated by Wellhausen (18) ranges from 1 to 5. One represents a degree of resistance in which there is no noticeable stunting of the inoculated plants and 5 represents extreme susceptibility, plants dying in 10 to 20 days after inoculation with a virulent strain of bacteria. Ratings 2, 3, and 4 are intermediate stages. Data on change in the proportion of virulent bacteria after passage, together with the rated resistance of each line, are given in table 7.

TABLE 7.—Change in proportion of virulent type colonies after passage through various maize lines of known resistance to *Phytophthora stewartii*, with average daily rate of change

Maize line or cross	Resistance of line ¹	Test 1, 19-day passage			Test 2, 21-day passage		
		Proportion of virulent type colonies present		Average daily rate of change (slope) ²	Proportion of virulent type colonies present		Average daily rate of change (slope) ²
		Initial	Final		Initial	Final	
		<i>Percent</i>	<i>Percent</i>		<i>Percent</i>	<i>Percent</i>	
GB134	5	41	28	-0.7	65	49	-0.8
887	5	41	24	-0.9	65	47	-0.9
Sto	5	41	33	-0.4	65	31	-1.6
WF	5	41	23	-1.0	65	45	-0.9
GF40	4	41	30	-0.6	65	56	-0.4
Mc	4	41	36	-0.3	65	60	-0.2
1339	3	41	36	-0.3	65	60	-0.2
Idt	3	41	43	+0.1	65	63	-0.1
Cl	2	41	19	-1.2	65	68	+0.1
BM	2	41	56	+0.8	65	33	-1.5
PR	1	41	52	+0.6	65	71	+0.3
MK	1	41	63	+1.2	65	83	+0.9
TR	1	41	54	+0.7	65	79	+0.7
OSF	1	41	54	+0.7	65	91	+1.2
MK × OSF	1				65	79	+0.7
OSF × GB134	1				65	79	+0.7

¹ Resistance as indicated: 1, Highly resistant; 2, resistant; 3, intermediate; 4, susceptible; 5, highly susceptible. (From Wellhausen (18).)

² See footnote 1, table 6.

It is again apparent that direction and rate of change in proportion of virulent bacteria during passage are dependent upon host resistance. There is a high correlation between host resistance and rate and direction of change in the bacterial population. The direction of

change in all lines of intermediate to low resistance (susceptible)—rates three, four, and five—is toward a lower proportion of virulent organisms. In resistant lines (rating one or two) the direction is toward a higher proportion of virulent organisms. One exception to this is noted—that of BM, a resistant early sweet corn inbred from the Black Mexican variety. This resistant line gave a selective reaction similar to that of a highly susceptible line. An explanation is not available at present.

Another test of host selection is to vary the virulence of the bacteria in a mixed culture used to judge selection rate and direction. Each of 7 bacterial stocks with virulence index ranging from 14 to 80 was mixed with a common avirulent stock (S15), passed through the same hosts (GB134 and OSF), and the direction and rate of change in the bacterial mixture determined.

All bacterial stocks were chosen both for a colony type and virulence that were very different from S15 stock. Of the stocks used, S15 and A14 have already been described. RM is a rough mucoid variant observed to arise from A14 during passage through the susceptible line. All other stocks were selections from the A88 experiment, the summarized data of which were presented in an earlier section. The technique of mixed culture selection is the same as that described earlier. S15 stock was used as the standard by which selection was judged, that is, the common stock with which all other strains were mixed. Results are presented in table 8. The degree of virulence was determined on GB797.

TABLE 8.—*Change in proportion of S15 type bacteria during 16-day passage through susceptible and resistant host and average daily rate of change*

Stock mixed with S15	Virulence index ¹ of pure culture	Initial proportion of S15 bacteria	Susceptible host, GB134		Resistance host, OSF	
			Proportion of S15	Average daily rate of change (slope) ²	Proportion of S15	Average daily rate of change (slope) ²
		Percent	Percent		Percent	
S15.....	30					
11.....	80	54	68	+0.9	39	-1.0
A14.....	76	57	68	+ .7	41	-1.1
93.....	63	50	59	+ .6	38	- .8
83.....	56	45	50	+ .3	40	- .3
RM.....	48	62	68	+ .4	57	- .3
47.....	57	42	44	+ .1	40	- .1
16.....	14	48	45	- .2	53	+ .3

¹ See footnote 1, table 1, and text p. 219.

² See footnote 1, table 6.

Direction and rate of change in the S15 type organism when passed through a common host (OSF or GB134) are both dependent upon the virulence of the bacterial strain with which it is mixed. The more extreme the virulence of the variant with which S15 is mixed, the more rapid the shift in the bacterial population. On passage through the susceptible line, selection was in favor of S15 when the variant with which it was mixed was more virulent; against S15 when the second strain was less virulent. In the resistant host, OSF, the converse was true. The experiment was not repeated since the results

are consistent and entirely comparable with the more extended S15-A14 mixtures.

These experiments, in which host resistance and bacterial virulence were varied, show that during host passage the direction and the rate of change of virulent and avirulent bacterial types in a population are dependent upon both host resistance and bacterial virulence. Where either resistance or virulence is held constant such a change is a direct function of the other; where both may vary, as would be true in a natural population, the change is due to an interaction of both variables.

NATURAL RATE OF MUTATION IN *PHYTOMONAS STEWARTII*

In preceding sections data have been presented showing that by host passage a change in virulence of bacterial populations is brought about, the direction and rate of this change being dependent upon host resistance and bacterial virulence. Where the initial inoculum originated from several colonies it has been shown that such cultures are composed of bacterial variants with different degrees of virulence (A88 experiment). For such cases selection explains the known facts of virulence change. When single-cell cultures are used (as was done with A14 stocks) some origin for the variability upon which selection works must be postulated. Since mutation is one known method for emergence of variability, it is important to ascertain whether mutations actually occur, their frequency, and the nature of such mutations.

All strains of *Phytomonas stewartii* used for mutation work were purified genetically by repeated single-celling. Agar colonies from such cultures were extremely uniform as to color, size, shape, and surface character. Bacteria from a single colony were used to inoculate 10 cc. of nutrient broth, a single, different colony being used for each tube of broth. These tubes were incubated at 27° C. for 18 to 20 hours, shaken repeatedly, diluted, and plated. Plates were incubated at room temperature for 3 days, then examined under a binocular dissecting microscope at 16 diameters. The total number of colonies was estimated by counting one-tenth of the area of a representative number of plates poured from each tube. Each observed variant was put into broth, replated, and the variant characters rechecked on this subplate before it was considered that a mutation had occurred.

For calculation of the maximum mutation rate the total number of colonies under observation was divided by the number of mutant colonies observed. This is considered a reasonable procedure since after a mutation arose, that mutant would increase in some ratio to the parent stock, the actual number of mutants observed depending upon the cell generation in which the mutation had originated and whether the mutation had originated in more than one cell. For the minimum mutation rate it has been assumed that a given mutation arose only once in a given tube, and repeated observations of that mutant merely indicate its later growth. For interpretation of these data it must be remembered that most variables tend to keep the mutation rate at a minimum.

Stock S15 and two color variants derived from this stock have been used to determine the mutation rate of characters distinguishable

from the normal strain by colony type and color. This stock was chosen because it was characterized by rough, raised, dark-yellow, small colonies that remained distinct and individual even when the colonies were crowded on agar plates. The small colonies of this strain allowed many more colonies per plate than any of the larger spreading types. Stock 101 is a pale-yellow derivative from S15, appearing to vary from the parent only in color. Stock 105 is a white derivative from S15.

TABLE 9.—*Natural mutation rate of three strains of *Phytophthora stewartii* after 18 hours' growth in nutrient broth*¹

Bacterial strain, number of tubes containing mutants, and rate of mutation	Tubes treated as units	Total colonies observed	Mutant colonies observed of type indicated							
			Dark yellow	Pale yellow	White	R2	R3	R4	Smooth	Sec-tored
	Number	Thousands	Number	Number	Number	Number	Number	Number	Number	Number
S15 (dark yellow, rough)	15	836		28	8	4	1		41	
Number of tubes in which mutation was observed				13	6	2	1		11	
Maximum rate of mutation per million individuals				33.3	9.6	4.8	1.2		49.0	
Minimum rate of mutation per million individuals				15.5	7.2	2.4	1.2		13.2	
101 (pale yellow, rough)	20	1,645	160		281	13		3	15	8
Number of tubes in which mutation was observed			17		5	11		2	9	8
Maximum rate of mutation per million individuals			97.3		170.8	7.9		1.8	9.1	4.9
Minimum rate of mutation per million individuals			10.3		3.0	6.6		1.2	5.5	4.9
105 (white, rough)	10	620				1				
Number of tubes in which mutation was observed						1				
Maximum rate of mutation per million individuals						1.6				
Minimum rate of mutation per million individuals						1.6				

¹ Complete tables in unpublished doctorate thesis. LINCOLN, R. E. RESISTANCE TO BACTERIAL WILT PATHOGEN OF MAIZE AND GENETIC HOST-PARASITE INTERACTIONS. 1939.

Mutation rates in the three stocks are given in table 9. Perhaps the most striking observation is the difference in mutation rates of the three stocks, the rate in the white stock being much lower than in the yellow stocks. Within the yellow stocks, usually the same characters were observed to vary, yet each stock yielded a type of variability not found in the other stock. The color mutations are interesting in showing that there are different intensities of yellow and that certain characters are more stable than others. Thus the mutation dark yellow to pale yellow and the reverse mutation—pale yellow to dark yellow—occur about equally often, whereas dark yellow mutates to white more readily than light yellow mutates to white. White is extremely stable, the mutation of white to yellow never having been observed. An examination of the different "rough" colonies shows that there are no fewer than four distinct and different types. These rough types have been designated as R1, R2, R3, and R4; R1 being the parental type in all three stocks used. The mutants classified as "smooth" probably are really several different smooth-type mutations rather than the same mutation in all cases. Because of the relation of smoothness to virulence, these smooth mutations are particularly significant.

The mutations are apparently stable from the time of origin. A pure culture of the variant type can be obtained directly from the variant colony, no type of segregation having been observed. Mutations arising as sectored colonies are apparently the same as mutations observed as entire colonies. Mutation in one character does not cause a mass mutation, for only one character has been observed to mutate at a time. Thus a colony having a color mutation is not morphologically different from the parent stock. This fact and the occurrence of reverse mutation suggests that the mutations observed are not "loss" type mutations such as would be occasioned by a chromosome deficiency. These variants may be point mutations in the genetic sense. Their mutation rates of 1 to 20,000 to 800,000 are not different from the known rate of many genes of higher organisms.

The term "mutation" rather than dissociation has been used because the observed variations are stable, heritable, and transmissible from parent to daughter cell. Such variant characters are stable under a variety of environments. The change from dark-yellow to pale-yellow colony color is one differing at least in quantity of pigment while the white form lacks all carotenoid pigment (unpublished data). Experimental evidence for mutation is furnished by Lincoln and Gowen,⁸ who obtained all the variants observed in this work as well as additional variation after treatment of this stock with X radiation. Rate of mutation after treatment with X radiation was much higher than the natural mutation rate given in this paper.

Since it was of particular interest to the interpretation of virulence change by host passage, several mutations observed to arise in pedigree cultures were tested for virulence. This was the only physiological test made of these variants. A single, unreplicated test was made. The virulence of each variant was compared with that of the type stock from which it arose (table 10).

TABLE 10.—*Virulence indices of mutants from R1 type stock*

Source and culture No.	Variant type	Virulence index ¹	Source and culture No.	Variant type	Virulence index ¹
From S15 stock (dark yellow):			From 101 stock (pale yellow):		
Parental type.....	R1.....	29	Parental type.....	R1.....	31
456.....	Smooth.....	48	409.....	Smooth.....	17
459.....	do.....	43	446.....	do.....	48
461.....	do.....	53	438.....	R2.....	25
462.....	do.....	36	414.....	R4.....	46
466.....	do.....	13	From 105 stock (white):		
473.....	do.....	47	Parental stock.....	R1.....	45
487.....	do.....	56	422.....	R2.....	34
454.....	White R1.....	49	480.....	R3.....	17
455.....	do.....	55			
401.....	Pale yellow R1.....	51			
407.....	do.....	31			
474.....	R2.....	36			

¹ See footnote 1, table 1, and text p. 219.

Stocks from which these mutations arose were all rough type (like S15). All mutations except those marked smooth are still in the rough phase. The increase in virulence discovered in most of the smooth mutations is important and is consonant with results in pas-

⁸ LINCOLN, R. E., and GOWEN, J. W. PRODUCTION AND RATE OF MUTATION IN *PHYTOMONAS STEWARTII* BY X RADIATION. Manuscript.

sage cultures. The exceptions need further study. It seems possible for virulence to be high or low in either the rough or smooth phase.

The demonstration of mutations in *Phytophthora stewartii* furnishes a source of variability great enough to account for the variation in virulence and colony type observed after host passage. This is particularly important in accounting for changes observed in single-cell cultures during host passage.

EXPERIMENTS FOR TESTING SEXUAL FUSION IN PHYTHOMONAS STEWARTII

If bacteria have sexual fusion then recombination and segregation of characters would be possible. This question is fundamental to an interpretation of the virulence changes in this investigation. Gowen and Lincoln⁹ have presented data on a technique which furnishes a certain type of information on this point. They grew in mixed broth cultures pure white and yellow strains of *Phytophthora stewartii*, plated from these mixed cultures at intervals, and replated from single colonies to observe possible segregation. If fusion and segregation were common, some of the colonies should segregate for both yellow and white colonies. There was no evidence of such segregation from their data. A possible limitation of their work is that only three repeatedly single-celled yellow cultures were tested against a white culture. If bacteria were of + and - strains the probability of testing unlike strains by this method would not be very high. Environmental conditions may also play an important part in reproductive processes. To correct for these two possible limiting factors and to collect more data by this technique, seven cultures that had been carried in stock without host passage or single celling were mixed with the white stock and passed through a resistant and a susceptible host. After 20 days' growth in the host, isolations were made and agar colonies plated (table 11).

TABLE 11.—Colonies after 20 days' host passage of mixed cultures of white and yellow bacteria plated to test for sexual fusion of white and yellow cells

Yellow strain with which white S15 variant was mixed	Single colonies plated				Plates showing yellow and white colonies from—	
	Susceptible host		Resistant host		Yellow	White
	Yellow	White	Yellow	White		
	Number	Number	Number	Number	Number	Number
A14.....	135	22	104	27	2	0
O23.....	218	25	160	15	2	0
A87.....	100	5	112	5	0	0
S15.....	157	18	103	12	1	0
FB32.....	120	10	84	10	4	0
D3.....	62	0	49	0	0	0
Total.....	792	80	672	69	9	0

A few yellow colonies showed segregation for white. Further plating from these yellow colonies did not show any segregation. This would be expected if the original colony started from two bacteria, one white, the other yellow. There is no indication of sexual fusion that may be determined by this method with any of the stocks used.

⁹ See footnote 7.

Such being the case, it seems unimportant whether a culture is derived from a single cell at the time the culture is taken or whether the culture is made from a group of cells one or two generations removed from a common cell since variation would be limited to such processes as mutation or somatic segregation.

DISCUSSION AND CONCLUSIONS

The opinion prevalent among bacteriologists is that host passage maintains or increases bacterial virulence. Recently Wellhausen (19) showed that the genetic constitution of the host is important in determining whether bacterial virulence is enhanced, maintained, or decreased.

This was verified in the present experiments by passing several stocks of *Phytomonas stewartii* through numerous lines of maize. Six stocks of bacteria were successively passed through inbred resistant or susceptible hosts. The results, with minor exceptions, prove that successive passage through susceptible lines decreases virulence, while passage through resistant lines increases the virulence of bacterial stocks.

More important, the virulence of 13 single-celled virulent stocks was decreased by passage through the susceptible host. Passage of these stocks through the resistant host maintained their virulence. The virulence of single-cell cultures changes in a manner comparable to that of stock cultures during host passage, although at a slower rate.

Virulence changes in mass cultures may be due entirely to host selection of variations known to be present. Quantitative proof of such differential host selection was obtained by inoculating susceptible and resistant hosts with mixtures of virulent and avirulent bacteria of known proportions, and at suitable intervals determining the direction and rate of change in the proportion of types. It is concluded that direction and intensity of selection for virulence change is largely dependent upon the resistance or susceptibility of the host.

Host passage of 13 single-cell cultures furnishes critical proof that adaptive variation does arise by some means and is subsequently selected.

The mutation rate for colony morphology and color was determined in nutrient broth on three bacterial strains. The mutation rate of characters studied was entirely comparable with mutation rates of genes determined experimentally in higher forms. Since evidence for sexual fusion in this species is entirely negative, mutation is probably the chief, perhaps the only, source of variation.

Mutation and natural selection within the host have been shown to be two important agencies in bringing about observed changes in bacterial virulence during host passage. In this respect the mechanisms for the evolution of bacterial virulence are not greatly different from those in higher organisms.

Considering the large number of individuals and generations possible in a few days of bacterial growth, combined with a mutation rate comparable to that in higher forms, it is entirely possible that selection processes in the microenvironment of the host are capable of effecting the observed changes in virulence of this pathogen.

Virulent bacteria kill the susceptible host in 10 to 15 days but only stunt the resistant host; avirulent bacteria stunt the susceptible host,

but in the resistant host soon become limited to a few early lesions. Successive passage of a bacterial stock through either the resistant or susceptible host effects a change in virulence of the bacteria to a point where that host is stunted noticeably but not killed. The host does not eliminate the parasite by outgrowing it. Once this virulence condition is reached, further host passage does not cause an appreciable change in virulence. Over long periods of time selection within the microenvironment of the host would be toward such a point of equilibrium.

SUMMARY

Six stock cultures of *Phytophthora stewartii* were successively passed through resistant and susceptible inbred lines of maize. Passage through the susceptible host decreased virulence, while passage through the resistant host increased virulence.

Change in virulence during passage was correlated with changes in colony growth on agar plates. Loss in virulence was always associated with a raised, firm type of colony but not necessarily with a change from the smooth to the rough phase. With an increase in virulence the colonies became more spreading, watery, and viscid. The change from rough to smooth and from smooth to rough was observed during passage.

Virulence of passage stocks could again be changed by reversing the host through which these stocks were passed.

Single-cell colonies from an old stock culture show that it is composed of many variants differing in virulence. Successive passage of this stock culture through susceptible maize decreased the proportion of virulent variants, while passage through resistant maize increased the proportion of virulent variants.

Virulence of a culture is a direct function of the proportion of virulent and avirulent organisms present in the culture.

As in stock cultures, virulent single-cell cultures lose virulence by passage through the susceptible host, while passage through the resistant host maintains high virulence.

Mixtures of virulent and avirulent bacteria of known proportions were inoculated into susceptible and resistant hosts, and the change in the proportions of bacteria followed at given intervals of time. There was differential selection for the avirulent type in the susceptible host and for the virulent type in the resistant host. With 1 exception this was true for 14 lines and 2 crosses of maize. Both rate and direction of change are functions of host resistance and bacterial virulence.

After 18 hours' growth in nutrient broth the mutation rate of colony color and colony morphology was determined on three bacterial strains. The mutation rate of these characters is of the same order as the known mutation rate of the genes in higher forms. The calculated mutation rate ranged from 1 in 20,000 to 1 in 800,000 individual cells.

The virulence of the mutations varied greatly from the virulence of the parent stock. Both increases and decreases in virulence were noted.

Sexual fusion of white and yellow strains of *Phytophthora stewartii* in the living host could not be demonstrated experimentally.

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RELATION OF THE NEAR-WILT FUNGUS TO THE PEA PLANT¹

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INTRODUCTION

Near-wilt of pea (*Pisum sativum* L.) was first described by Snyder (5).³ Later Snyder and Walker (7) defined the causal organism of the disease as *Fusarium oxysporum* Schlecht. f. 8 Snyder. Snyder (6, p. 458) also reported that *F. vasinfectum* Atk. var. *pisi* Van Hall, one of the organisms involved in the complex St. John's disease in Europe, is synonymous with *F. oxysporum* f. 8. Appel (1), many years before Snyder described near-wilt in the United States, found that germination was reduced when healthy seed of field peas was inoculated with a spore suspension of *F. vasinfectum* var. *pisi*. Snyder and Walker (7) reported that small rootlets of near-wilt-infected pea plants may show evidence of decay, and in some cases may be rotted away, but that in general the root and foot of the plants are free of any conspicuous decay of the cortex. There may be, however, conditions of infection which favor invasion of the cortex, and symptoms of root rot attended by a slight discoloration of the cortical tissues may be detected. Virgin and Walker (10) found that there was a distinct coincidence between the appearance of near-wilt and the beginning of the blossoming period. Starr (8), working in Minnesota with a *Fusarium* wilt of pea, found the causal organism to be seed-borne and the fungus to be present in the seed coats but not in the cotyledons.

The purpose of the investigation reported in this paper was to study the mode of entrance of the near-wilt fungus into the host, the course of the pathogen after penetration, its effect on the host, and the possibility of the organism being seed-borne.

MATERIALS AND METHODS

The following varieties of peas were used in this study: Wisconsin Perfection, Wisconsin Early Sweet, Dwarf White Sugar, Alaska, Prince of Wales, Penin, Senator, all very susceptible to near-wilt, and Rogers K, a variety possessing decided resistance to the disease. The fungus used throughout the investigation was from a culture isolated by the writers and identified as *Fusarium oxysporum* f. 8 by W. C. Snyder of the University of California.

Peas were germinated aseptically; and when the radicles were about an inch long, they were placed in contact with the near-wilt fungus which was growing on potato-dextrose agar. These peas were left

¹ Received for publication July 31, 1939. Joint contribution from the Departments of Plant Pathology of the University of Idaho and of the University of Wisconsin. Research Paper No. 182, of the Idaho Agricultural Experiment Station.

² Appreciation is expressed to Wilbur Schroeder, formerly a fellow in Plant Pathology, University of Idaho, for his assistance on certain parts of this investigation.

³ Italic numbers in parentheses refer to Literature Cited, p. 248.

for different lengths of time before being fixed and sectioned. Other pea radicles were placed in contact with the fungus for various lengths of time and then transferred to a moist chamber and suspended on a mesh screen. At suitable intervals they were removed, placed in a fixative, and were later sectioned. Peas were planted in sterilized sand which had previously been infested with the near-wilt fungus, and when the seedlings were 5 to 8 days old, they were removed and plated out on acidified potato-dextrose agar in order to determine the location of the fungus. Stems from plants that were dying from the disease were also fixed and sectioned.

Seeds of the variety Wisconsin Early Sweet which had been surface-sterilized were planted in sterilized sand, but before they were covered they were sprayed with a heavy suspension of macroconidia of the near-wilt fungus. After 7 days the affected seedlings were removed, washed thoroughly, and plated out on potato-dextrose agar without being surface-sterilized.

The fixative used, consisting of two solutions, A and B, was a combination of Bouin's fluid and Karpechenko's modification of Navashin's solution. Solution A, containing 100 cc. of water, 1 gm. of chromic acid, and 4 cc. of glacial acetic acid, was saturated with picric acid. Solution B consisted of formalin. Two parts of solution A were mixed with 1 part of solution B just before using.

The process of dehydration and infiltration with paraffin was carried out according to the schedule given by Rawlins (3, p. 21), in which cedar oil was used following a series of alcohols of various concentrations up to 95 percent. For mature pea stems, which were quite woody, butyl alcohol was substituted for ethyl alcohol after the material had been brought to 70-percent concentration. Sections were cut 8 to 15 μ in thickness. The material was stained with Delafield's haematoxylin followed by orange G in 95-percent alcohol.

For seed-transmission studies, peas were grown in near-wilt-infested soil. The vines were supported on trellises to prevent the pods from coming in contact with the soil, and seed was harvested from plants that survived. Seed was also collected from diseased plants grown on naturally infested soil. Both lots of seed were planted in soil known to be free from the near-wilt fungus. For isolation of the organism from the seed the latter was sterilized by placing in 70 percent alcohol for 30 seconds and then in a solution of sodium hypochlorite (B. K. 1-10) for 10 minutes. The seeds were then soaked in sterile water for 4 hours to facilitate separating the seed coat from the cotyledons, and the various parts were then placed on plates of potato-dextrose agar.

EXPERIMENTAL RESULTS

PENETRATION

The near-wilt fungus was found to penetrate the young pea seedling most commonly at the root tip and the cotyledonary node. Penetration occasionally took place at various points along the root and epicotyl and sometimes at the growing point of the latter. No noticeable differences were observed in the penetration of the very susceptible variety, Wisconsin Perfection, and the resistant variety, Rogers K.

Repeated platings of 5- to 8-day-old seedlings grown in near-wilt-infested sand showed the location of the fungus at the cotyledonary node and the root tip. In order to make sure that the fungus did not enter at other points and when plated out on agar grow out at the cotyledonary node, some of the seedlings were cut in small pieces and plated. Many of the seedlings showed infection only at the cotyledonary node (fig. 1, *A*), others at the root tip, and some at both places (fig. 1, *B*). Occasionally in some of the seedlings plated, the fungus was found at the tip of the epicotyl (fig. 1, *C*), and in others it was found to grow out along the epicotyl a short distance above the cotyledonary node (fig. 1, *D*).

The fungus accumulated in a mass on the root-cap cells (pl. 1, *A*), where the hyphae penetrated between the cells and soon entered the embryonic region. Intercellular preceded intracellular penetration in this region, but the reverse order frequently occurred in older tissues. After the organism had gained entrance into the meristematic region, it progressed with little difficulty through the region of elongation into the xylem vessels of the young root. Very often the entire root tip was so thoroughly invaded that it was impossible to distinguish the host tissue from the fungus mass.

The near-wilt fungus was able to penetrate the root at points other than the root tip and cotyledonary node. When a mass of mycelium accumulated at some point along the root, the hyphal threads soon penetrated the epidermis and cortex both intercellularly and intracellularly (pl. 1, *B*). The fungus progressed up and down the cortex as it made its way inward to

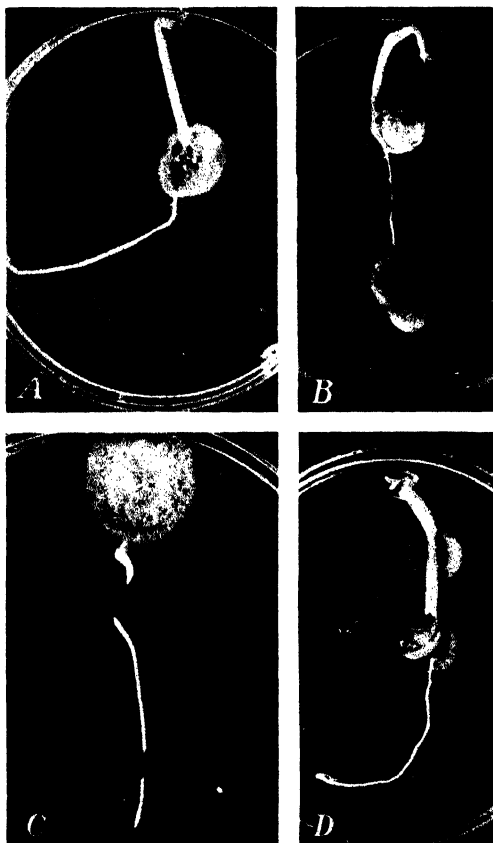


FIGURE 1.—Platings of seedlings grown in near-wilt-infested sand: *A*, 5-day-old Rogers K seedling showing location of organism at the cotyledonary node; *B*, a similar seedling showing location of infection at both the root tip and the cotyledonary node; *C*, 5-day-old Wisconsin Perfection seedling showing location of fungus at the growing point of the epicotyl; *D*, a similar seedling showing location of infection in the epicotyl and at the cotyledonary node.

the stele (pl. 1, *C*), where it entered the xylem elements by way of the pits.

Seedlings resulting from the seed that was sprayed with a suspension of macroconidia were rotted at the cotyledonary region and for some distance below it. The near-wilt fungus grew out abundantly from the rotted area, indicating that the organism had thoroughly penetrated the cortex. No other organisms were present.

Infection in lateral roots took place similarly to that in primary roots. Penetration was not observed where the emerging secondary root ruptured the cortex and epidermis.

SUBSEQUENT DEVELOPMENT OF THE FUNGUS AND THE EFFECT ON ITS HOST

After initial penetration the organism soon made its way both intercellularly and intracellularly to the stele of the root. Some development of the fungus occurred in the cortex, occasionally causing complete disintegration and a consequent seedling rot. Upon entering the stele the organism was confined there until the advanced stage of the disease developed. The fungus was found in both large and small xylem vessels, and it was through these that the organism made its upward advance from the point of infection (pl. 1, *D* and *E*). Progress from one vessel to another took place through the pits. Many of the xylem vessels became completely plugged with hyphae, and microconidia were frequently observed in some of the larger vessels.

The fungus was found to progress the entire length of the stems in many of the susceptible varieties of peas. When stems from wilted plants were placed in moist chambers for 60 hours, the fungus grew out profusely from them (fig. 2). A number of stems were cut and separated at each internode to make sure that no upward growth of the organism occurred while in the moist chamber. However, the fungus was still found to occur throughout the length of many of the stems, indicating that the fungus had already advanced through them before being placed in the moist chamber.

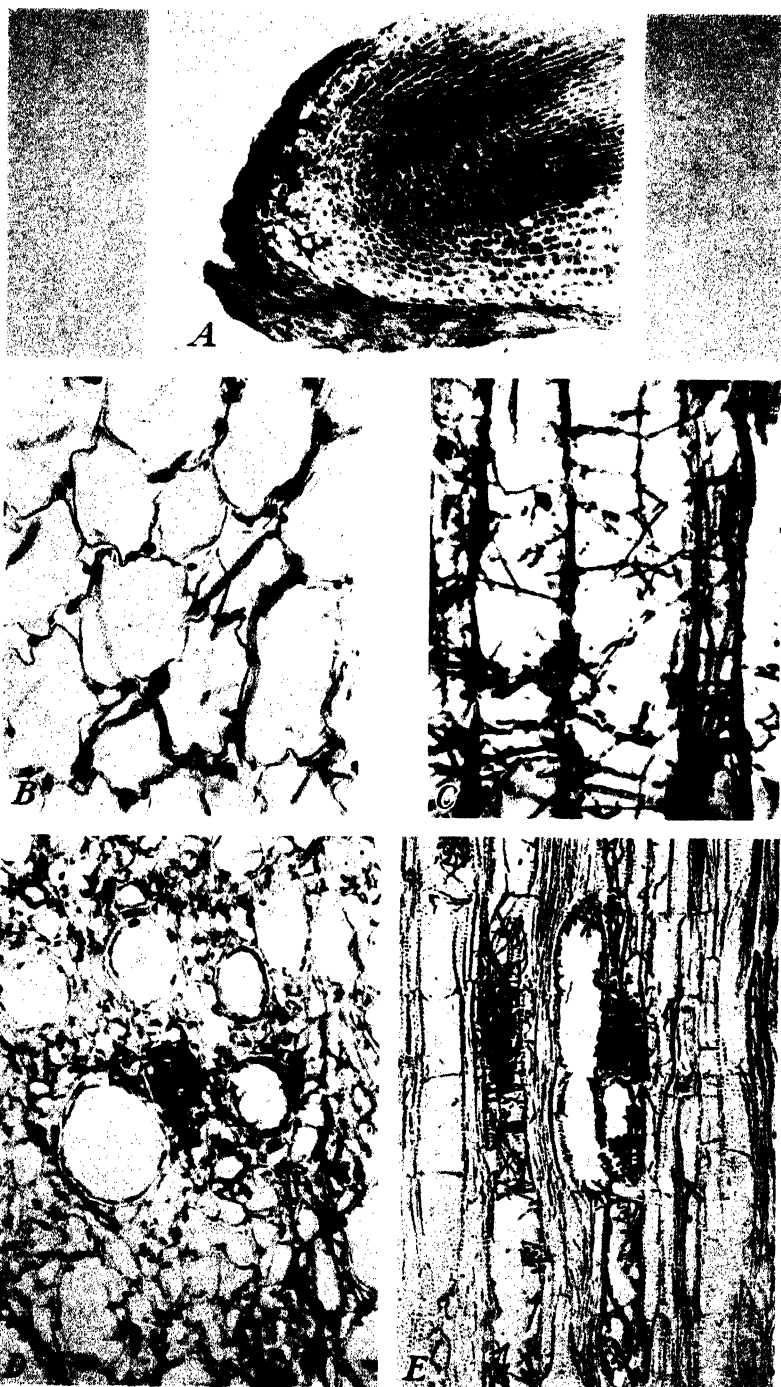
In the case of resistant Rogers K and some promising hybrids of which it was one of the parents, the fungus did not advance beyond the eighth node when grown on near-wilt-infested soil.

SEED TRANSMISSION

Since the fungus was found to advance from the root through the entire length of the stem of susceptible pea varieties, and because of the fact that the disease does not ordinarily kill the plant until the middle of its development or later, usually when pods are setting, it seemed highly probable that the fungus might reach the seed through the vascular system.

EXPLANATORY LEGEND FOR PLATE 1

A, Longitudinal section of an infected root tip of a Rogers K pea plant, showing accumulation of mycelium around it and intercellular penetration; *B*, cross section of cortex of seedling root, showing fungus in both intercellular and intracellular relation to the host cells; *C*, longitudinal section of cortex of seedling root, showing a considerable amount of the fungus present, infection having taken place along the surface of the root; *D*, cross section through the first internode of a plant that died at the time it was setting pods; *E*, longitudinal section through the first internode of the same plant. Note abundance of mycelium in certain of the vessels.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.



FIGURE 2.—Near-wilt-diseased vine of Dwarf White Sugar placed in a moist chamber for 60 hours in order to promote fruiting of the pathogen on the surface as an indication of the extent of its invasion. The fungus extended almost to the tip of the stem, which was 3 feet 4 inches in length.

Seeds of several varieties of peas were collected from near-wilt-diseased plants grown on naturally infested soil in the field. These plants generally had set one or two pods, although many of the pods failed to develop seed. The seed coats were separated from the cotyledons of a number of these seeds when plated out on potato dextrose agar. The near-wilt fungus was found in the seed coats and in the cotyledons, but not necessarily in both in the same seed (fig. 3). The results of the isolation studies are shown in table 1. Several isolates were also obtained from seed produced by susceptible plants grown on near-wilt-infested soil in the greenhouse.

Seed collected from two varieties of peas, Wisconsin Perfection and Prince of Wales, grown on a naturally infested field in Wisconsin



FIGURE 3.—Seeds from near-wilt-infected pea plants plated on potato-dextrose agar. The seed coats and cotyledons were separated before plating; A, Fungus present only in seed coat; B, fungus present only in cotyledon; C, fungus present in both seed coat and cotyledon.

were planted at Moscow, Idaho, on soil where near-wilt had never been observed. Out of 140 plants of Wisconsin Perfection, 16 became diseased with near-wilt, and out of 656 plants of Prince of Wales, 10 were diseased. Fifty-four pea crosses, mostly backcrosses from small-podded, somewhat resistant lines to near-wilt-susceptible, large-podded varieties, were made in the greenhouse on near-wilt-infested soil. Most of the plants on which the crosses were made set one or two pods before dying from near-wilt. The 54 crosses furnished about 140 seeds, which were planted the following spring in the plots at Moscow. Four of the resulting plants became affected with the disease. These cases of near-wilt were the only ones found in the plot, which covered about one-fifth of an acre.

TABLE 1.—Results of plating seeds collected from near-wilt-diseased plants

Variety	Seeds tested	Seeds infected
	Number	Number
Prince of Wales.....	70	2
Wisconsin Perfection.....	70	1
Penin.....	141	21
Senator.....	50	1

The results of the isolation experiments leave little doubt that the diseased plants developed from infected seed produced by susceptible plants grown on near-wilt-infested soil.

DISCUSSION

Penetration of the root tip of pea plants by the near-wilt organism is quite similar to penetration of root tips of China-aster plants by *Fusarium conglutinans* Wr. var. *callistephi* Beach as described by Ullstrup (9), and the penetration of cabbage plants by the cabbage yellows organism (*Fusarium conglutinans* Wr.) reported by Smith and Walker (4). The near-wilt organism differs, however, from these strictly vascular parasites in that it may also invade the cortex extensively. In this respect it is similar to the watermelon wilt organism (*Fusarium niveum* Erw. F. Sm.). No explanation is offered of why the fungus invades the cotyledonary region so readily, unless it could be that the organism in contact with a germinating seed grows over its surface and immediately attacks the cotyledonary region when the young seedling breaks through the seed coat. What conditions are necessary for the organism to cause a rot are not definitely known, but it does seem that an abundance of mycelium must be present on the surface of the seedling to initiate it. Results quite similar to those reported by Appel (1) were obtained when the seed was sprayed with a suspension of macroconidia of the near-wilt fungus.

The ability of the organism to travel the complete length of the stem of susceptible pea varieties is an outstanding difference between this disease and the wilt disease described by Linford (2), who never found the wilt fungus (*Fusarium orthoceras* App. and Wr. var. *psii* Linford) within two nodes of the lowest blossoms of susceptible varieties. It has been shown in this investigation that penetration takes place equally well in Wisconsin Perfection and Rogers K, but that the fungus does not advance nearly so far up the stem in the latter as in the more susceptible varieties. No morphological differences were observed which would account for this difference. Perhaps some substance is present in Rogers K which slows down the development of the fungus, and it therefore could progress only so far before the plant matured.

It seems logical to assume that certain varieties are more liable than others to produce infected seed when grown on infested soil. Early-maturing types, such as Alaska, often mature before the organism can reach the pods, whereas in later maturing ones like Perfection and Penin the organism has a much longer time to travel approximately the same distance to reach the seed. It is doubtful if the organism could reach the seed of very tall varieties of peas by way of the vascular system except under very favorable conditions. Although only a small percentage of seeds carry the fungus, there is ample opportunity for it to become widely disseminated. Thus, it is possible that the near-wilt disease may at some time become a more serious menace to the pea industry than it has up to the present.

SUMMARY

The near-wilt fungus (*Fusarium oxysporum* Schlecht. f. 8 Snyder) makes its entry into the pea plant at almost any point along the root and epicotyl of young seedlings, the root tip and cotyledonary node being the most common points of entry. No difference in penetration of Wisconsin Perfection, a very susceptible variety, and Rogers K,

a somewhat resistant variety, was observed. Under certain conditions the fungus was able to produce a definite seedling root rot.

The progress of the fungus through the root and stem was largely confined to the xylem vessels. The organism was able to travel the complete length of stems of many susceptible varieties. In the case of Rogers K and some hybrids of which Rogers K was one of the parents, the fungus failed to advance nearly so far up the stem as in the susceptible varieties.

The organism advances into the seeds of diseased plants through the vascular system. The fungus was found both in the seed coat and cotyledon. Dwarf, late-maturing, susceptible varieties are much more likely to produce infected seed when grown in near-wilt-infested soil than are early-maturing varieties. Seed infected by the near-wilt organism will produce diseased plants.

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PRELIMINARY STUDIES OF THE NUTRITIVE REQUIREMENTS OF THE EUROPEAN CORN BORER¹

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INTRODUCTION

Much research conducted on the European corn borer (*Pyrausta nubilalis* (Hbn.)) in relation to potential host plants, number of generations, breeding of imported parasites, and especially borer-resistant strains of corn has emphasized a growing need for accurate knowledge of the nutritive requirements of the insect.

After a certain strain or variety of any plant has been proved to possess some resistance to the attack of an insect, the possibility of breeding this resistant quality into new strains of the plant that are adapted to different localities and purposes would appear far more encouraging if the plant breeder could be furnished definite knowledge of the insect's nutritive requirements. A working knowledge of the inherent characters or factors which constitute or contribute to the resistance of a particular variety of plant to attack by a certain insect would permit the immediate elimination of many varieties or strains of the plants from extensive but futile tests of resistance. Definite information concerning the physiological effect of nutrients on an insect might also aid in forecasting the response of the insect's parasites when the food plant of the insect is growing in a different environment, or the magnitude that an infestation of the insect might be expected to assume on a new or different host plant.

During the spring of 1937 tests were begun at the Toledo, Ohio, research laboratory to study the nutritional requirements and limitations of the European corn borer with special attention to its ability to feed on and assimilate the tissues of different food plants. The results of these tests are reported in the present paper.

METHODS, TEST MATERIAL, AND EQUIPMENT

Each experiment for testing the effect of nutrients on the borer was replicated four times, and the material used in each replication was infested with approximately 100 eggs. For a period of 25 days the test larvae were supplied with fresh food every fifth day. Thirty days after infestation the surviving larvae were weighed and isolated for further observations on development, survival, pupation, and emergence. Although the percentage of pupation prior to diapause among ostensibly single-generation borers has been considered negligible, the percentage of larvae in these tests that pupated without diapause varied considerably with the nature of the nutritive sub-

¹ Received for publication November 20, 1939.

² Grateful acknowledgment of assistance is made to W. A. Baker, in charge of corn borer research, under whose leadership these studies were initiated, and to A. M. Vance, under whose general supervision the tests were conducted.

stance supplied them. Thus, in many instances the pupation of a portion of the larval population reduced the number of larvae available for weighing, and for this reason the weights of larvae as recorded from such populations may not be entirely comparable with those representing populations in which no pupation occurred. No pupae were weighed.

Supplementary tests for enzymes present in the digestive tracts of corn borer larvae were conducted subsequent to these feeding experiments in an effort to gain some fundamental knowledge concerning the insect's digestive capacities.

FOOD MATERIALS

Food materials tested were selected to represent distinct types of physical structure, differences in stage of plant maturity and in moisture content, different chemical constituents, and different strains of corn that had proved either resistant or susceptible under field tests as reported by Patch and Bottger³ and Marston and Dibble (6).⁴

Before infestation the food materials were washed and cut or broken into portions which exposed a comparatively large amount of feeding surface to the borer. The fragments of food usually were laid close together to provide an environment to satisfy the borers' thigmotropism.

Proximate composition of vegetable foods tested was assumed from analyses by Chatfield and Adams (2) and from detailed analyses of sugars in sweet corn at different stages of maturity by Culpepper and Magoon (3). The interpretations involving the chemical constituents of material are based on such assumptions.

Fresh vegetables such as green beans, green peas, lettuce, and cauliflower were purchased from markets as needed. Five successive plantings of four different types of corn and of five other kinds of crop plants provided additional materials for both field and laboratory tests.

The kinds and portions of the plants tested are shown in table 1.

TEST INSECTS

Fall-collected corn borer larvae, all from one source, were isolated in glass shell vials and kept in cold storage at a temperature of 40° F. and a relative humidity of 70 to 100 percent. Moths emerging from this stock produced the eggs used for infesting all materials included in the tests.

LABORATORY CONDITIONS

A room-size incubator kept at a constant temperature of 80° F. and a relative humidity of 70 to 100 percent provided satisfactory conditions for emerging moths, oviposition, and the rearing of larvae on the foods tested. A metal rack permitted the stacking of the rearing trays within a comparatively small space and at the same time allowed free circulation of air which was maintained by the continuous operation of a 12-inch electric fan in the room.

PRODUCTION OF EGGS

A slight modification of the mating and ovipositing cage described by Bottger and Kent (1) was used. Essentially this consisted of a

³ PATCH, L. H., and BOTTGER, G. T. INVESTIGATIONS OF THE VARIETAL RESISTANCE OF FIELD CORN TO THE EUROPEAN CORN BORER IN 1935. U. S. Bur. Ent. and Plant Quarantine E-416, 11 pp. 1937. [Mimeographed.]

⁴ Italic numbers in parentheses refer to Literature Cited, p. 257.

3½-inch cardboard ice-cream container fitted top and bottom with an 18-mesh copper screen. Its inner wall was lined with waxed paper, on which the moths readily oviposited.

From 5 to 10 female moths and a like number of males were confined in each of these cages and kept in the incubator for mating and oviposition. Once every 24 hours the sheets of waxed paper bearing the egg masses were removed from the oviposition cages. The paper was then cut into pieces so that each piece bore a single egg mass. These were placed in terra-cotta dishes, covered with a glass plate, and returned to the incubator room for incubation of the eggs. The glass cover permitted the frequent observation of embryonic development, which was essential because the eggs had to be removed just prior to hatching for ease in counting and for insurance of a complete hatch after they were placed on food material.

INFESTATION OF FOOD MATERIALS

The eggs were incubated until the head capsules of the contained larvae became sufficiently pigmented to be readily visible, at which time groups of approximately 100 eggs were counted and placed on food in rearing trays, where hatching usually occurred within a few hours. The number of eggs failing to hatch was negligible.

REARING TRAYS

Two types of rearing trays were used in the laboratory tests. The one most generally employed was that described by Mathes (7). It consisted of a rectangular wooden frame fitted with an 80-mesh copper-screen bottom and a glass top.

For some of the more concentrated foods which contained little fiber, a terra-cotta dish 7 inches in diameter and 2 inches deep was found practical. A satisfactory lid was provided by a round piece of 80-mesh screen of the same circumference as the inside of the dish about one-fourth inch from its top. The slightly flared sides of the dish served as a shoulder on which the rough edges of the screen cover rested.

ENZYME TESTS

The entire digestive tracts from 25 hibernating larvae were ground with glycerin in a mortar to form a tissue suspension to make each test for an enzyme.

The methods of enzyme analysis used were the microchemical methods reported by Swingle (8).

RESULTS

The percentages of survival and pupation and the weights of test larvae of the European corn borer reared on 33 samples of plant material are presented in table 1. Each number is the average of four replications.

These data were analyzed by the method of the analysis of variance described by Fisher (4). The required difference for significance between treatment means was calculated from the remainder variance and from the value of t obtained from Fisher's table of t , in which the number of degrees of freedom applicable to this problem for a probability of 0.05 was used. The significance of the correlation

coefficient was determined by computing t from the correlation coefficient and the number of pairs of observations on which the correlation was based, less 2. The probability of such a value was then ascertained from Fisher's table of t .

TABLE 1.—Effect of certain nutrients on survival, weight, and pupation of European corn borer larvae, arranged in decreasing order of survival

Plant	Portion of plant fed larvae for 30 days	Survival of test larvae	Average weight per larva	Larvae pupated 1—		
				30 days after hatching	40 days after hatching	60 days after hatching
		Percent	Milligrams	Percent	Percent	Percent
Sweet corn (<i>Zea mays</i>)	Kernels	65.3	66.6	21.7	38.6	41.9
Green beans (<i>Phaseolus vulgaris</i>)	Pods and fruit	51.6	96.0	9.6	12.5	12.5
Popcorn, Japanese hull-less	Internodes	47.1	92.9	8.8	10.8	11.4
Dent corn, A × Tr	do	43.5	100.1	17.2	23.6	27.0
Sweet corn, bantam	do	41.1	120.1	13.5	15.3	16.0
Dent corn, Hy × R4	do	33.0	78.3	11.4	15.9	19.7
Lettuce (<i>Lactuca sativa</i>)	Leaves	32.0	83.2	0	1.6	1.6
Flint corn, maize Amargo	Internodes	30.1	124.4	1.9	2.9	2.9
Green peas (<i>Pisum sativum</i>)	Pods and fruit	21.2	92.6	3.5	3.5	4.7
Sorghum (<i>Holcus sorghum</i>)	Leaves and stem	18.4	102.8	7.9	14.4	14.4
	Internodes	14.6	67.4	10.0	10.0	10.0
Sweetpotato (<i>Ipomoea batatas</i>)	Roots	12.5	43.6	2.0	3.9	3.9
Sunflower (<i>Helianthus</i> sp.)	Leaves and stem	10.3	25.0	0	0	0
Smartweed (<i>Polygonum</i> sp.)	do	8.9	62.2	2.7	5.0	5.0
Cocklebur (<i>Xanthium</i> sp.)	do	8.1	71.1	3.3	10.0	10.0
Table beet (<i>Beta vulgaris</i>)	Roots	7.4	80.5	0	0	0
Dent corn, A × Tr	Leaves	6.9	73.1	13.8	13.8	13.8
Flint corn, maize Amargo	do	6.9	53.2	17.0	17.9	17.9
Sunflower	Sections of stalk	5.4	31.7	0	0	0
Giant ragweed (<i>Ambrosia trifida</i>)	Leaves and stem	5.4	46.0	0	0	0
Asparagus (<i>Asparagus officinalis</i>)	Young shoots	5.0	53.6	0	0	0
Banana (<i>Musa sapientum</i>)	Fruit	4.1	62.8	0	0	0
Cauliflower (<i>Brassica oleracea botrytis</i>)	Flower	3.9	73.2	11.1	11.1	11.1
Sweet corn, bantam	Leaves	3.9	32.0	18.8	18.8	18.8
Dent corn, Hy × R4	do	2.5	55.8	0	0	0
Popcorn, Japanese hull-less	do	2.4	28.7	² 42.9	² 42.9	² 42.9
Hemp (<i>Cannabis sativa</i>)	Leaves and stem	1.9	26.1	0	0	0
Potato (<i>Solanum tuberosum</i>)	Tubers	.7	51.1	² 33.3	² 33.3	² 33.3
	Leaves and stem	.5	17.8	0	0	0
Dent corn, Hy × R4	Tassels	0	0	0	0	0
Dent corn, A × Tr	do	0	0	0	0	0
Soybean (<i>Glycine max</i>)	Leaves and stem	0	0	0	0	0
Table beet	Leaves	0	0	0	0	0
Required difference for significance at odds of 19 to 1.		13.1	34.1	32.1	0	0

¹ Based on numbers of larvae surviving.

² Extremely low larval survivals resulted in populations too low for reliable comparison with percentages of pupation occurring among the other populations.

SURVIVAL

On the basis of the required difference of 13.1 percent for odds of 19 to 1, the 65.3 percent of larvae which survived on a diet of sweet corn kernels was significantly greater than the percentage which survived on any other food tested.

The survival of 51.6 percent of larvae nourished on green beans was next highest to the survival on sweet corn kernels and was significantly higher than any survival of 38.5 percent or less. The survivals of 47.1, 43.5, and 41.1 percent on the internodes of popcorn A × Tr dent corn, and bantam sweet corn, respectively, were significantly higher than any survival of 28 percent or less. The 43.5 percent survival of larvae nourished on the internodes of A × Tr was significantly higher than the 30.1 percent survival on the internodes of the flint strain, maize Amargo, but not significantly higher than the 33

percent survival on the dent Hy \times R4 internodes. However, the survival of 47.1 percent on popcorn internodes was significantly higher than the survival on either of the more resistant strains Hy \times R4 and maize Amargo.

The 32-percent survival of larvae on lettuce compared favorably with that on the internodes of the two resistant strains of corn, all of which were significantly higher than any survival falling at or below 17 percent.

Survivals of 21.2 and 18.4 percent on pods and fruit of green peas and on leaves and stem of sorghum, respectively, while significantly higher than any falling at or below 5.3 percent, represented the lower brackets of survivals indicative of satisfactory nutrition.

Corn leaves (except from maize Amargo and from the strain A \times Tr), corn tassels, beet leaves, hemp plants, potato plants, potato tubers, and soybean plants were foods on which either no survival resulted or an extremely low one (significantly lower than survival of 17 percent or more).

WEIGHTS

It is apparent that larvae reared on most of the corn internodes, green beans, and green peas attained greater weights than did those reared on other foods. The average weights of 124.4 and 120.1 mg. per larva nourished on maize Amargo and bantam sweet corn internodes, respectively, were significantly greater than the average weight per larva of 78.3 mg. for those reared on the internodes of Hy \times R4.

The average weights of larvae reared on each of the five kinds of corn leaves (except those of A \times Tr), sunflower plants, and hemp and potato plants were significantly less than the average weights of larvae reared on each of the five kinds of corn internodes (except those of Hy \times R4), sorghum plants (leaves and stem), green beans, and green peas.

Statistically there is a high correlation between percentage of survival and average weight per larva 30 days after hatching. The correlation coefficient of 0.635 was calculated from 29 pairs of observed values.

PUPATION

Statistical analyses of the percentages of pupation are of little value because of the widely different populations of surviving larvae on which the analyses were based. However, excepting the instances where extremely low larval survivals resulted in populations too small for reliable comparison, relatively high percentages of pupation are associated with relatively high percentages of survival and weights of mature larvae.

The 38.6 percent of larvae reared on sweet corn kernels which had pupated 40 days after hatching exceeded the pupation for all comparable groups, again placing this nutrient in a unique position, when compared with other nutrients, in relation to pupation.

ENZYMES

Table 2 summarizes the results of tests for enzymes in the digestive tracts of corn borer larvae. The positive reaction of tissue suspensions with sucrose, olive oil, fibrin, and peptone suggests the borer's ability to digest sucrose, fat, and proteins. The pepsin reaction may have been affected by the presence of excess acid in the solution. Pepsin

is active only in a strongly acid solution, and this necessitates a more careful adjustment of the pH value than available equipment would permit. However, the reaction indicating the presence of erepsin was so pronounced that its presence could scarcely be doubted. In the presence of erepsin, peptones are broken down into amino acids, indicating the borer's ability to digest these derivatives of protein matter. The borer's inability to digest starch, maltose, and lactose is indicated by the negative reaction of the tests for amylase, maltase, and lactase.

TABLE 2.—*Summary of results of tests for enzymes in the tissues from walls of the entire digestive tracts of European corn borer larvae*

Enzyme sought	Substrate used	Tests made	Enzymatic reaction
		<i>Number</i>	
Amylase.....	Cornstarch.....	4	Negative
Maltase.....	Maltose.....	1	Do.
Invertase.....	Sucrose.....	4	Positive.
Lactase.....	Lactose.....	1	Negative.
Lipase.....	Olive oil.....	2	Positive.
Pepsin.....	Fibrin.....	4	Do.
Erepsin.....	Peptone.....	4	Do.

DISCUSSION

VEGETABLES

The data indicate that the nutritive elements available in immature sweet corn kernels were better adapted to the borer's requirements, when measured by survival and pupation, than those in any other food tested. Green beans, lettuce, and green peas were fairly satisfactory nutrients from the standpoint of survival and larval weight, but they did not affect the metabolic acceleration of the test insects as expressed in percentage of pupation nearly so much as did the corn kernels.

The low survivals of larvae on the other vegetables, accompanied by low weights in some instances and either low or no pupation in all instances, may have been due to mechanical resistance to attack offered by the physical structure of the plants, such as hard epidermis, or to retention of too much or too little moisture under laboratory conditions, deficiency in certain chemical constituents, or the occurrence of these constituents in a form not readily digested by the borer.

Considering the borer's favorable physiological reaction to nutrients which are relatively high in glucose, such as immature sweet corn kernels (3), compared with its reaction when fed foods such as beet leaves, in which practically all sugar present is sucrose, and potato tubers, which are high in starch, it seems probable that the character of the carbohydrate constituents of food may be of real importance in determining its nutritive value to the borer. On the basis of these indications, it seems logical to assume that glucose fulfills the insect's nutritive requirements far more effectively than does either sucrose or starch. Failure to obtain a favorable growth response of larvae to predominantly starchy food materials is probably due to the inability of the insect to digest starch, as was indicated by the negative reaction of the tests for amylase, the starch-splitting enzyme.

CORN INTERNODES

Relatively high survivals of larvae resulted from infestations on the internodes of all strains of corn and were accompanied by favorable rates of development in most instances, probably because of the comparatively high percentage of digestible carbohydrate constituents in green corn internodes. The differences in survival and weight of larvae between populations nourished on internodes of different strains indicate that the nutrients present which are best adapted to the borer's requirements vary considerably in amount as between different strains. That these nutrients do vary was further substantiated by physical differences in the excrement of the test larvae feeding on internodes from different strains of corn. Excrement of larvae which were feeding on internodes of the resistant strain Hy \times R4 was coarse-textured and retained the original color of the food. In contrast, the excrement from larvae feeding on internodes of the susceptible strain A \times Tr was fine-textured and greatly changed in color, a condition which would indicate a much more complete digestion.

The low survivals and weights of larvae confined on corn leaves are attributed to causes of a mechanical rather than a nutritive nature, and to the fact that the leaves became very dry during the interval between feedings. Since the thick portion of the midribs and a small area of the leaf and sheath adjacent to the ligules were the only portions of the leaves which appeared to satisfy the positive thigmotropism of the borer, it seems probable that the majority of larvae were unable to find an environment conducive to their normal activities and subsequent development. Both etiolated and chlorophyllaceous leaf tissues were supplied the larvae in these tests.

The fact that no larvae survived on the tassels of the two strains of dent corn was contrary to expectations, because the tassel is one of the most favorable points of entrance, and its availability has been commonly associated with high borer survivals in the field. While newly hatched larvae appeared to feed normally on the tassel spikelets, any feeding observed on tassels under test after the larvae were 5 days old was confined to the main stem, and as soon as that source of nutrition was eliminated, either by larval feeding or by desiccation, the larvae died without any apparent attempt to feed on the spikelets. Corn tassels dried rapidly after they were placed in rearing cages, and this may have caused a large portion of the larval mortality.

The close similarity between the carbohydrate constituents of leaves, tassels, and stalks of green corn plants, as shown by the analysis of Latshaw and Miller (5), makes it unreasonable to assume that carbohydrate nutritive values were responsible for the marked physiological differences in test larvae nourished on them.

MISCELLANEOUS CROP PLANTS AND WEEDS

The total mortality of larvae confined to soybean plants appears to have been due largely to the mechanical barrier afforded by the profuse pubescence peculiar to all aerial portions of these plants. Many dead first-instar larvae were observed which had become lodged between the bristly hairs composing the pubescence.

Thick layers of coarse fibrovascular bundles were observed to surround the pith of sunflower, hemp, and giant ragweed plants and are believed to have contributed to the resistance of such plants, because relatively low survivals and weights of larvae were associated with

plants in which the fibrovascular character was prominent. High water content of the sunflower stems apparently prevented the normal tunneling and feeding of the few borers which were successful in gaining entrance through the bristly pubescence and hard epidermis.

Neither the physical structure nor the nutritive qualities of cocklebur, smartweed, giant ragweed, or potato plants could be considered favorable to the establishment and development of the borer, on the basis of its physiological reactions to any of them. The physical changes which occurred in these plants under laboratory conditions doubtless contributed to their unsatisfactory nutritive qualities.

With the sorghums, weights of larvae reared on internodes were significantly lower than of those reared on leaves and stems of much younger plants, thus indicating that the changes in these plants, probably both physical and chemical, which occur with maturity affect the borer adversely.

ENZYMES

The absence of amylase (starch-splitting enzyme) in the borer's digestive tract as indicated by these tests is offered as a possible explanation of low survivals and weights of larvae which resulted when they were confined to starchy food materials such as mature potato tubers. The presence of protein-splitting enzymes such as pepsin and crepsin is in keeping with favorable survivals and growth rates of larvae reared on foods such as green beans, green peas, and green corn kernels, all known to be relatively rich sources of protein. A pronounced positive reaction for sucrase leaves little doubt of the presence of this enzyme in the walls of the borer's digestive tract. Obviously the more favorable growth rate of larvae on foods relatively rich in glucose than of larvae reared on foods rich in sucrose, but containing little glucose, cannot, on the basis of these tests, be attributed to the nonexistence of sugar-splitting enzymes.

SUMMARY AND CONCLUSIONS

During the spring of 1937 a study of the nutritional requirements of the European corn borer (*Pyrausta nubilalis* (Hbn.)) was undertaken at Toledo, Ohio. The food materials tested were selected to represent distinct types in physical structure, stage of maturity, moisture content, chemical constituents, and both resistant and susceptible strains of corn. Their proximate composition was assumed from analyses by Chatfield and Adams and Culpepper and Magoon.

Moths that emerged in the laboratory from one lot of corn borer larvae collected in the field the previous fall supplied the eggs and resulting larvae used for all the tests. A room-size incubator kept at 80° F. and a relative humidity of 70 to 100 percent was used for the emerging moths, oviposition, and the rearing of larvae on the foods.

As measured by statistically significant differences in percentage of survival and by the relatively high percentage of pupation of test larvae, the sweet corn kernels satisfied the borer's nutritive requirements better than any other material.

Comparatively high percentages of larvae survived on green beans, lettuce, and green peas, and the comparatively high weights of larvae nourished on these foods indicated that they provided satisfactory nutrition.

Internodes of corn in general provided satisfactory nutrients for the borer. The nutritive qualities of corn internodes, as measured

by differences in survivals and weights of corn borer larvae nourished on them, varied considerably as between the strains tested.

Extremely low survivals resulted from infestations induced on potato plants and mature tubers, hemp, and sunflower plants. Soybean plants, corn tassels, and beet leaves were the only green-plant food materials tested in the laboratory on which no larvae survived, although in the case of corn tassels mortality may have been due to the poor condition of the food.

Food materials rich in glucose fulfilled the borer's nutritive requirements far better than did those high in either sucrose or starch.

Physical characters of plants which were associated with low survivals and weights of larvae were profuse pubescence, thick epidermis, and the greater number and coarse nature of fibrovascular bundles, especially in the peripheral region of the stems.

Excessive moisture in such succulent foods as young sunflower plants and potatoes apparently interfered with the tunneling habits of the insect. Insufficient moisture to satisfy physiological requirements of the insect and physical structure of the plant tissue incompatible with the positive thigmotropism of the insect probably contributed more to larval mortality on such foods as corn leaves and tassels than any deficiencies in nutritive elements.

A positive correlation was found between survival of larvae and their average weights 30 days after hatching.

Supplementary tests for enzymes in the cellular tissue of the digestive tracts of hibernating larvae indicated the presence of both sucrose- and protein-splitting enzymes, but no enzymes capable of hydrolyzing starch were found.

Metamorphic development of the insect was directly affected by nutrition. This observation suggests a possible explanation for the variation in number of generations, within a climatic range, on the basis of difference in the nutritive value of plants available in different environments.

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PHYSIOLOGICAL STUDIES OF PLASTID PIGMENTS IN RINDS OF MATURING ORANGES¹

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INTRODUCTION

Growers of citrus fruits have long been aware that rind color is not necessarily an index of dessert quality. Early-maturing varieties of oranges are quite edible before they have lost their green pigment. In later varieties, such as Valencia, this color change occurs long before the fruits become suitable for consumption and they may regreen during the normal harvest period. Fruits of late varieties sometimes fail to lose their green color because of shade or other local conditions and may pass into senility and finally drop from the tree without having shown the characteristic orange color. The latter aspect becomes of increasing commercial significance along with increasing age of the grove.

For many years it has been customary to remove the green rind color, under conditions indicated above, by the use of ethylene. The process whereby the fruit loses this green pigment (chlorophyll) is termed "degreening," whether it is permitted to take place on the tree or whether it is hastened by the use of ethylene. Frequently difficulties are encountered, as a result of which the fruits, though legally mature, cannot be properly degreened for the market. Although many improvements have been made in the mechanical equipment employed in degreening fruit, little is known concerning physiological changes taking place in the rind during the degreening process. The present work was inaugurated in the hope that a detailed investigation of these processes might help solve some of the present-day problems in the industry.

REVIEW OF LITERATURE

While a number of investigators have identified the carotenoid pigments occurring in citrus fruits, only a few have studied the pigment transformations during degreening of the fruit or the influence of ethylene on this process. Gardner (4)³ demonstrated the presence of a chlorophyll-decomposing enzyme in the rinds of satsuma oranges. Stahl (11) reported a slight increase in carotenoid pigments in orange rinds during maturation. Chace (1) stated that "the changes produced by ethylene gas are solely those which would have been brought about by nature in a somewhat longer time." Crocker et al. (3) suggested that since ethylene is a product of the metabolism of plant

¹ Received for publication March 31, 1939.

² The writers are indebted to D. F. Fisher, of the Division of Fruit and Vegetable Crops and Diseases, for helpful suggestions in planning these investigations, to C. J. Thompson, of the same Division, for constructing and assisting in adapting the Clifford photometer, and to P. A. Clifford, of the Food and Drug Administration, U. S. Department of Agriculture, for supplying specifications for the light filters. Appreciation is expressed also to Ward B. Matthews, Doris Prohlitt, and Robert Bunch, of the Division of Fruit and Vegetable Crops and Diseases, for assistance in the analytical work.

³ Italic numbers in parentheses refer to Literature Cited, p. 267.

organs it might act as a fruit-ripening hormone. Michener (6) stated that the effects of ethylene on growth are to be explained not as direct effects of ethylene alone, but as effects of ethylene on a growth hormone. Lynch (5) formulated the theory that ethylene acts as a coenzyme in the ripening processes of fruits.

MATERIALS AND METHODS

Studies were conducted during the 1936-37 citrus season at Orlando, Fla., and continued during the 1937-38 season at the Arlington Experiment Farm, Arlington, Va. The fruit used during the second season was shipped by express from Florida.

Parson Brown⁴ oranges were obtained from what is known as the Carney grove at Oklawaha, Marion County, one of the original plantings of this variety. This grove is located in the northern fruit section of the Florida citrus belt, on "high hammock" soil originally supporting a growth of hardwood trees. The rootstock was sour orange. The Parson Brown orange was introduced by J. L. Carney, former owner of this grove, the budwood having been selected from what was considered the best of five promising seedling trees in the grove of "Parson" Brown, about 30 miles south. This variety is one of Florida's early oranges, in season during October and November.

Pineapple⁴ oranges were collected in the Crosby-Wartman grove at Citra, Marion County. The grove is situated in a "low hammock," and there is a limestone formation a few feet under the soil. The original growth was mostly hardwood. This grove is nearly at the northern limit of citrus production on a commercial scale. The trees were budded in place on wild sour orange trees found growing in the uncleared forest. Budwood for the trees in this grove came from the original Pineapple orange tree,⁵ produced in the grove of J. B. Owens, about 5 miles from Citra. The season for pineapple oranges is December to February.

Valencia⁴ oranges were obtained from a grove belonging to the Tampa Forwarding Co., 2 miles southeast of Dundee, in Polk County. The grove is located in the "ridge section" of Florida, on what is locally known as "high pine sandy soil." The trees are on rough lemon rootstock. The Valencia season extends from about March to June.

Temple⁶ and tangerine⁷ oranges were all collected from commercial groves near Orlando.

Ten trees in each of the groves described above were reserved for the experimental work. Samples of Parson Brown and Pineapple oranges were collected every 3 weeks, and Valencia samples were taken every 4 weeks. Only 2 of the 10 trees were reserved for the few samples taken after the commercial picking dates.

Before an intelligent investigation can be made of pigment transformations induced in citrus rinds by ethylene treatment, it is essential to know what changes take place in nature. The most obvious effect of degreening is the loss of chlorophyll. However, an accurate appraisal of changes in carotenoid content cannot be made without recourse to chemical analysis.

⁴ Parson Brown, Pineapple, and Valencia are varieties of *Citrus sinensis* (L.) Osbeck.

⁵ The writers are indebted to W. J. Crosby, of Citra, Fla., for these historical data.

⁶ Origin unknown; supposed to be a natural hybrid with *Citrus nobilis* var. *deliciosa* (Tenore) Swingle as one of the parents.

⁷ *Citrus nobilis* var. *deliciosa*.

Thirty representative fruits were selected for individual samples. The fruits were peeled with a sharp scalpel, care being taken to remove both albedo and flavedo without rupturing the juice sacs of the pulp. The peel was ground in a food chopper. The procedure followed in the extraction of the plastid pigments was essentially that recommended by Schertz (10). Duplicate 20-gm. samples of the ground peel were covered with a 1-percent solution of sodium carbonate (Na_2CO_3) and ground to a paste with clean quartz sand in a mortar. The sample was then transferred quantitatively to a Büchner funnel, pure acetone being used to rinse the mortar. The paste was extracted with acetone until the extracts were colorless. The combined extracts were taken up with ether, treated with a little 1-percent sodium carbonate solution, and washed repeatedly with water until the ethereal solution was free from flavones. Five milliliters of absolute methanol saturated with potassium hydroxide (KOH) was added to the extract, which was shaken and then stored in the refrigerator overnight. The next day the chlorophyll, which had been saponified by the treatment described, was washed out with water. Schertz's precautions for complete separation of the chlorophyll from the carotenoid pigments were observed by rewashing each fraction until the particular solvent was colorless. The solution was made up to volume at 20° C., and its chlorophyll content was determined colorimetrically.

The ethereal solution of carotenoid pigments was dehydrated with anhydrous sodium sulfate and evaporated to dryness at a temperature below 50° C. At this place in the procedure Peterson's method (9) for the separation of carotene from xanthophyll was substituted for that of Schertz. The petroleum-ether fraction (cryptoxanthin, carotene)⁸ was made up to 100 ml., and the methanol fraction was made up to 200 ml. The pigments were determined colorimetrically.

Colorimetric determinations were made with a Clifford (2) photometer.⁹ The instrument was adapted for this purpose by employing a red light filter for chlorophyll and a blue filter for the carotenoids. The former consisted of dark-shade pyrometer red, Corning No. 241, transmitting from 630 m μ up. The blue filter consisted of a combination of Corning violet No. 511 and Noviol shade A No. 038, transmitting at approximately 458 m μ . The photometer wedge was standardized with solutions of crystals of chlorophyll, of beta-carotene, and of xanthophyll.¹⁰ The same zero point could be used for each of the three solvents by compensating with clear microscope slides, thus eliminating the necessity for changing the position of the wedge in determining the three pigments. Successive readings on the same solution could be replicated within 1 or 2 mm. on the scale with a 2-inch cell. Accurate readings on pigments could be made with chlorophyll solutions as dilute as 1 part per million and with the carotenoids as dilute as 1 part in 20 million. All solutions were made up to volume at 20° C., and the readings were made as soon there-

⁸ In the determination of plastid pigments in green leaves it is customary to designate the petroleum-ether fraction of carotenoids as carotene and the methanol fraction as xanthophyll. Recently Zechmeister and Tuzson (12) have reported that cryptoxanthin is much more abundant than carotene in the fruits of the Seville orange (*Citrus aurantium* L.). In view of this report, these two carotenoid fractions will be referred to in this paper as the petroleum-ether fraction and the methanol fraction, even though the color standards employed were beta-carotene and leaf xanthophyll, respectively.

⁹ Munsey (8) has employed the Clifford photometer for carotene determinations and suggests its use for chlorophyll and xanthophyll readings.

¹⁰ Especially prepared for standardization by F. M. Schertz.

after as practicable, although tests made at 20°, 25°, and 30° indicated that no significant errors would result from temperature fluctuations in this range. The photometer offers the additional advantage that when it is used there is no need to employ standard solutions for comparison after the wedge has once been standardized.

RESULTS

PROGRESSIVE CHANGES IN PLASTID PIGMENTS

The progressive changes in the plastid pigments of the rinds of Parson Brown, Pineapple, and Valencia oranges are shown in figure 1.

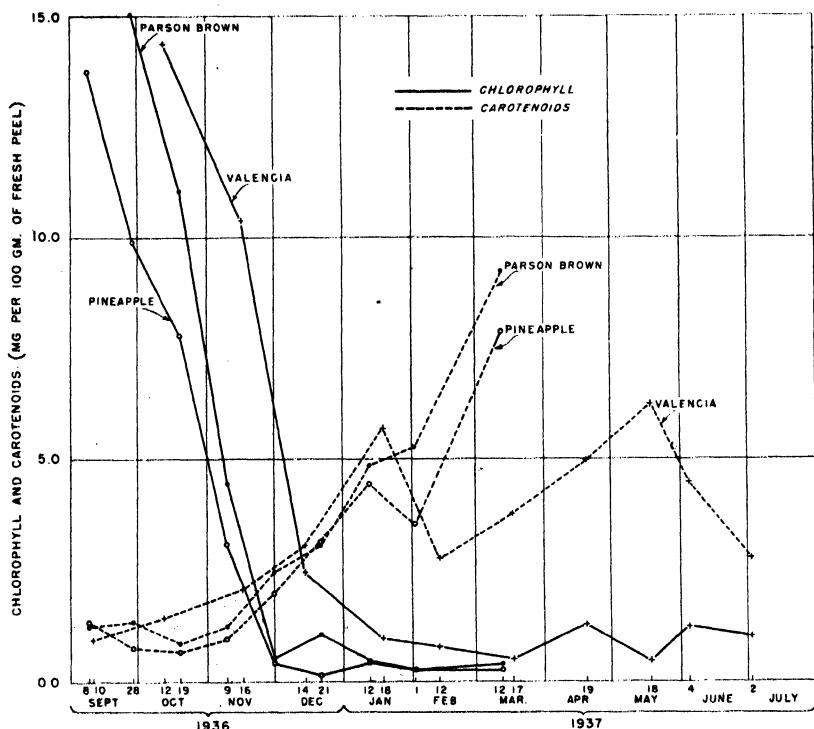


FIGURE 1.—Progressive changes in chlorophyll and carotenoids in orange rinds.

The initial samples were collected just before the fruits showed the first signs of losing their green color. From this time on the loss of chlorophyll proceeded rapidly and rather steadily. The curve for chlorophyll in Valencia oranges never reached the zero point, because of the regreening of the fruits in the spring when the trees began making new growth. Fluctuations are doubtless due to sampling errors, because all fruits on the same tree do not regreen to the same extent and these were random samples selected for uniformity of size.

From these results it appears that Parson Brown and Pineapple oranges never completely lost their chlorophyll. Whether this is the case is not definitely known. After the fruit has attained full color on the tree there is still a minute amount of pigment that is determined

as chlorophyll by the method employed. It is not known whether this is chlorophyll that is being formed as a result of the new growth and occurs in quantities too minute to be detected with the eye, or whether it is a pigment isolated along with chlorophyll but not detectable until the chlorophyll has all disappeared.

It is interesting to note that the carotenoid pigments in the rinds of all three varieties began increasing rather early in the fall and before the rind had lost its green color. The regularity of the curves is broken by the February samples. Pineapple and Valencia varieties showed a loss in carotenoids at this time, and the Parson Brown variety showed only a slight increase. However, carotenoid values reached a second peak in March for Parson Brown and Pineapple varieties and in May for Valencia. The March samples constituted the last for the early and midseason varieties, because this was long after the picking season for Parson Brown and rather late for Pineapple oranges. The Valencia oranges showed lower carotenoid content in the peel on each of the sampling dates subsequent to May. In this variety, too, the fruit had passed its most marketable stage at the time of the last sampling.

The next question arising was whether deeper colored fruits, such as tangerine and Temple oranges, show a similar increase in carotenoid content of the rinds during maturation. These particular fruits were sampled at but two intervals, (1) when mature green and (2) when full-colored (degreened on the tree). The results will be found in table 1. The green fruits of these two varieties contained a greater quantity of carotenoids than similar fruits of the *Citrus sinensis* type (Parson Brown, Pineapple, and Valencia). Nevertheless the fully colored fruits showed still higher quantities of carotenoids. The tangerine oranges doubled their carotenoid content in attaining full color while fruits of the Temple variety more than tripled theirs.

TABLE 1.—*Plastid pigment content of the rinds of mature green and full-colored tangerine and Temple oranges*

Variety and stage	Date	Total chlorophyll ¹	Total carotenoids ¹
Tangerine:		<i>Milligrams</i>	<i>Milligrams</i>
Mature green.....	Oct. 31, 1936	14.583	4.083
Full-colored.....	Dec. 9, 1936	.000	8.925
Temple:			
Mature green.....	Dec. 7, 1936	3.525	2.732
Full-colored.....	Jan. 6, 1937	.000	9.500

¹ Expressed as milligrams per 100 gm. of fresh peel.

EFFECT OF ETHYLENE ON PLASTID PIGMENTS

Mature green fruits were given ethylene treatment under conditions that simulated commercial conditions as far as possible. A coloring room was equipped to maintain a constant temperature of 27.8° C. and a relative humidity of 88 percent. The air in the room was circulated by means of a fan, and ethylene was introduced at the rate of 0.532 cubic feet per 24 hours. Carotenoid determinations were made on a sample before ethylene treatment, and on comparable samples after various periods of treatment. The results appear in table 2. In commercial practice the ethylene treatment is rarely applied for more than 2 days and under no circumstances would the application

standards of maturity. The carotenoids in the peel of this variety dropped in February, rose in May, and dropped again in July. From the results with both early and late varieties, it is apparent that maximum yellow color is not always correlated with maximum quality.

The effect of ethylene on green orange rinds is to stimulate degreening. Carotenoids in the peel showed no significant changes as a result of ethylene treatment. Frequently the exposure of oranges to the ethylene treatment for several days in the fall appears to enhance the yellow color. A close inspection reveals that this effect is produced by deeper-colored yellow splotches distributed irregularly over the surface of the rind. Chemical analyses indicated that there was no increase in actual carotenoid content.

The above results for oranges are quite different from those previously reported for limes, lemons, and grapefruit (7). In these lighter-colored citrus fruits there was a decrease in carotenoids as they degreened and this decrease occurred whether the fruit was degreened by ethylene or was permitted to degreen on the tree. With all types of citrus fruit investigated, however, the effect of ethylene appeared to be merely to stimulate chlorophyll decomposition and no change was produced in the fruit that would not have occurred in nature in a somewhat longer time.

It is interesting to note that in the earliest samples of oranges the methanol fraction (xanthophyll) of the carotenoids predominated, whereas the final samples showed a predominance of the petroleum-ether fraction (cryptoxanthin, carotene). In the unevenly colored fruit, resulting from unequal exposure to sunlight, the deeper-colored fruit or the deeper-colored side also showed a predominance of the petroleum-ether fraction. Inasmuch as cryptoxanthin is the only plant carotenoid beside the carotenes that has been reported as a precursor of vitamin A, it would be interesting to determine whether the petroleum-ether fraction also shows similar variation in the edible portion of the fruit.

SUMMARY

Adaptation of the Clifford photometer for quantitative determinations of the plastid pigments is described.

Periodic analyses were made on the rinds of early (Parson Brown), midseason (Pineapple), and late (Valencia) Florida oranges from the time they were mature green until long after their marketing season. The results show that as the chlorophyll decreased the carotenoids increased and continued to increase after the chlorophyll had disappeared.

Fully colored tangerine and Temple orange rinds contained a greater quantity of carotenoid pigments than the rinds of the mature green fruit.

Ethylene treatment of mature green oranges stimulated decomposition of chlorophyll without any significant effect on the carotenoid pigments.

In the samples taken when the fruits were mature green the methanol fraction (xanthophyll) predominated in the carotenoids in the rind. Later, when the fruit had attained its highest carotenoid content, the petroleum-ether fraction (cryptoxanthin, carotene) was much higher than the methanol fraction.

Pineapple oranges selected from the northeast side of the tree in the spring owe their superiority in color over those on the southwest side to the higher petroleum-ether fraction of the carotenoids. This is true also when there is a difference in color of the two sides of the same fruit.

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PRODUCTION OF EPINASTY BY EMANATIONS FROM NORMAL AND DECAYING CITRUS FRUITS AND FROM *PENICILLIUM DIGITATUM*¹

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INTRODUCTION

It is common knowledge that mature green citrus fruits will eventually lose their green color if held long enough in air at room temperature. When mature green Key limes are shipped to market they frequently arrive at destination fully colored (yellow) and with the receptacles loosened in a manner similar to that occurring when citrus fruits are held in coloring rooms. Furthermore, it has been frequently observed in the Horticultural Field Laboratory at Orlando, Fla., that when a high percentage of decay occurs in stored lots of mature green oranges the surviving fruits degreen at an accelerated rate.

The present investigation was undertaken for the purpose of determining the reason for the aforementioned physiological phenomenon.

REVIEW OF LITERATURE

A considerable number of plant tissues have been shown to evolve a gaseous substance that causes epinasty in test plants such as the potato or tomato. It was suggested by Botjes (1)³ and by Huelin (8), in 1933, that this gaseous substance might be ethylene. During recent years several investigators have conducted chemical tests that serve to prove the correctness of this assumption. In 1933 Kidd and West (10) reported experiments which showed that the physiologically active substance in apple vapors was a hydrocarbon, and the work of Smith and Gane (13) during the same year indicated that the substance might be an olefine or some complex hydrocarbon. In 1936 Elmer (4), analyzing the volatile products of apples which are absorbed by fuming sulfuric acid, concluded that the hydrocarbon was ethylene rather than one of its homologues. Hansen and Hartman (7), in 1935, as well as Denny (3), in 1938, eliminated the possibility of butylene and propylene by filtering the gaseous emanations through 87-percent sulfuric acid and removed any acetylene that might be present by absorbing the gas in mercuric nitrate and liberating the pure ethylene by adding hydrochloric acid. As early as 1934, however, Gane (5) had rather conclusively proved that apples evolve ethylene. The method consisted of absorbing the gaseous emanations in bromine and converting the ethylene dibromide thus formed into crystalline diphenyl-ethylene-diamine. Niederl, Brenner, and Kelley (12) and Niederl and Brenner (11), working with bananas, confirmed

¹ Received for publication November 20, 1939.

² The writers wish to acknowledge the assistance of A. Peyton Musselwhite in conducting these experiments.

³ Italic numbers in parentheses refer to Literature Cited, p. 277.

Gane's results by determining ethylene as silver and copper acetylide.

It is reasonably certain, therefore, that ethylene is the gas involved in the production of epinasty by the gaseous emanation from plant tissue. The list of materials reported as showing positive results includes the fruits of the apple, peach, pear, plum, banana, avocado, loquat, tomato, cantaloup, squash, and eggplant, as well as seed, flowers, shoots, and leafy stems of certain other plants.

Cousins, as reported by the Jamaica Department of Agriculture (9, p. 7) in 1910, stated that gaseous emanations from oranges accelerated the ripening of bananas; to the writers' knowledge, this is the only work suggesting that ethylene might be evolved by citrus fruits. Elmer (4) reported no inhibition in the growth of potato sprouts when exposed to the emanations of oranges, bananas, or decayed apples.

Not a great deal is known concerning the physiological properties of emanations from fungi. Gane (6) found that the growth of pea seedlings was inhibited by the atmosphere from baker's yeast when the culture was grown aerobically. Although this toxic substance resembled ethylene in that it could be removed by absorption in bromine water, it did not stimulate the ripening of bananas. Denny (2) lists the following lower organisms as not producing epinasty: Baker's yeast, growing on sugar; *Rhizopus nigricans* Ehr., in potato-dextrose agar; and the fully expanded pileus and stalk of two species of mushrooms.

MATERIALS AND METHODS

The following citrus fruits were used in these investigations: Fully colored fruits of Parson Brown, Pineapple, Valencia, and seedling oranges (*Citrus sinensis* (L.) Osbeck); tangerines (*C. nobilis* var. *deliciosa* (Tenore) Swingle); partly colored and fully colored grapefruit (*C. grandis* (L.) Osbeck); Villafranca lemons (*C. limonia* Osbeck) and green and fully colored Key limes (*C. aurantifolia* (Christm.) Swingle). All of these fruits, except the Key limes, were grown in central Florida, either in the vicinity of Orlando or within a radius of 40 miles. Key limes were produced in Key Largo and shipped to Orlando by express. In most cases the fruit was placed under experimental conditions as soon as it arrived at the laboratory. When this was impracticable, it was stored overnight either in an open shed or in the basement of a building having no outlets for illuminating gas. All experiments were conducted in these rooms in order to eliminate possible contamination with ethylene from illuminating gas, coloring rooms, or other sources.

The following pure cultures of fungi were studied: *Diplodia natalensis* Ev., *Diaporthe citri* (Fawc.) Wolf; *Penicillium digitatum* (Fr.) Sacc.; and *Alternaria citri* N. B. Pierce. These organisms are among those most frequently isolated from fruit decaying in storage.

The technique employed for testing the citrus fruits was as follows: Potassium hydroxide pellets were first placed on the bottom of a 5-gallon wide-mouthed bottle to absorb the carbon dioxide produced by the fruit during the course of the experiment. The test plant was then supported above the potash by means of a coarse wire-mesh platform. The fruits were stacked around the test plant, which consisted of a potted tomato, potato, or sunflower plant, or a potato cutting in water. The jar was closed by means of a metal top and a

rubber gasket. The numbers of fruits used were as follows: 100 limes, 24 oranges or lemons, and 6 to 12 grapefruits. When decaying fruit was used the number was reduced to 5 or 10.

Two types of decaying fruit were used: (1) Fruit inoculated with the green mold organism (*Penicillium digitatum*) and (2) fruit showing stem-end decay caused by *Diplodia natalensis* and *Diaporthe citri*. Oranges were inoculated with green mold by washing the fruit with mercuric chloride and implanting the spores in the sides of the fruit by means of a sharp scalpel. Attempts to inoculate fruit with stem-end decay organisms were abandoned because of occasional contaminations with *Penicillium* after the skin was once ruptured. Instead, sound fruit was held in closed containers until definite and unmistakable stem-end rot lesions appeared, which were the result of natural infection. Sound fruit was selected from the same lot for the control, thus insuring identical treatments for both series. Cultures made of the decayed fruit at the close of the experiment showed approximately 50 percent of *Diplodia natalensis* and 50 percent of *Diaporthe citri*.

The fungi tested were grown on potato-dextrose agar in 1-liter Erlenmeyer flasks. The general scheme for the experiment was similar to that followed for the fruit. The metal top of the jar was removed and replaced by the open, inverted culture flask. The flask was fitted within the neck of the jar with two large rubber bands and weighted down with a brick.

RESULTS

Epinasty of potato leaves was produced by all of the citrus fruits tested, i. e., fully colored Parson Brown, Pineapple, Valencia, seedling, and tangerine oranges; partially and fully colored grapefruit; Villafraña lemons; and green and fully colored Key limes (fig. 1, A and B). During the winter when the temperature was between 15° and 20° C., approximately 12 to 24 hours was required to produce definite symptoms on the test plants. During the spring months when the temperature was around 25°, epinasty appeared within 4 or 6 hours. Similar results were obtained when potato cuttings, tomato plants, or sunflower plants were used for tests.

In several experiments potato plants were exposed separately to emanations from the peel, pulp, seeds, and juice of grapefruit. Only those plants exposed to the pulp showed epinasty. Typical oil injury was produced by the peel. Identical results were obtained with the peel, pulp, juice, and seeds of oranges. The results are shown in figure 1, C.

When grapefruits or oranges were inoculated with *Penicillium digitatum*⁴ and tested for ethylene emanations it was found that decaying fruit produced epinasty more quickly than did the normal or sound fruit. The same was true when oranges showing stem-end rot lesions were used. Typical results with green mold decay are shown in figure 2. In figure 2, A, the photograph was made after 3 hours. At that time the plant in jar c, which was surrounded by decaying

⁴ In an intraoffice communication, E. M. Harvey, of this Division, reported that he noted epinastic responses of potato plants when these were placed in the rooms of a commercial lemon-storage building, but because of the abundance of the decayed fruit present he suspected that ethylene might be evolved by the rot-producing fungi rather than by the fruit itself.

fruit, was the first to show epinasty. After 5 hours the plant with the sound oranges (fig. 2, *B*, jar *b*) showed epinasty also. The results with stem-end rot are shown in figure 3. In figure 3, *A*, the photograph was made after 4½ hours. Again, the plant in jar *c*, with the decaying fruit, showed epinasty, while the plant with the sound fruit



FIGURE 1.—Epinasty of potato leaves caused by emanations from oranges and limes and from various grapefruit tissues. *A*: *a*, Control; *b*, exposed to Parson Brown oranges; *c*, exposed to Pineapple oranges; *d*, exposed to Valencia oranges. *B*: *a*, Control; *b*, exposed to mature green Key limes; *c*, exposed to fully colored Key limes. *C*: *a*, Control; *b*, exposed to pulp of one grapefruit; *c*, exposed to seeds of one grapefruit; *d*, exposed to juice of one grapefruit.



FIGURE 2.—Epinasty of potato leaves caused by sound and decaying Valencia oranges. A: a, Control; b, exposed to sound oranges; c, exposed to oranges inoculated with *Penicillium digitatum*. Photographed after 3 hours. B: Same as A, but photographed after 5 hours.

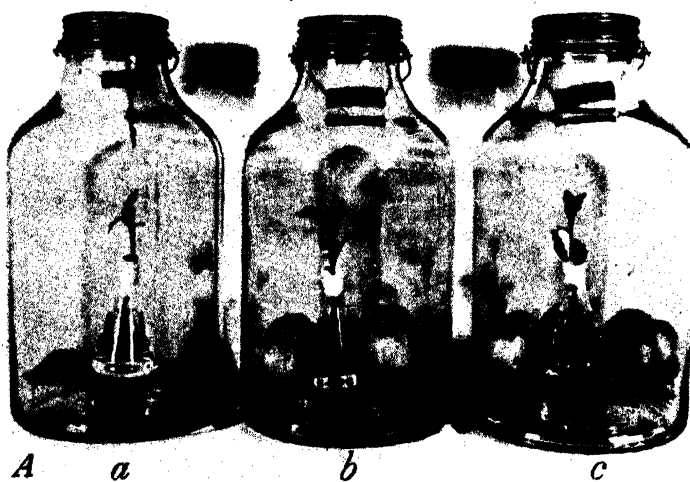


FIGURE 3.—Epinasty of potato leaves caused by emanations from sound and decaying Valencia oranges. A: a, Control; b, exposed to sound oranges; c, exposed to oranges showing stem-end decay. Photographed after 4½ hours. B: Same as A, but photographed after 16 hours.

(jar *b*) still appeared normal. The photograph in figure 3, *B*, was made after 16 hours. At this time symptoms of epinasty also appeared in the plant exposed to the sound fruit (jar *b*), although the effect was not so pronounced as with the decaying fruit (jar *c*). In figure 4, *A*, are shown the effects of the emanations from sound and decayed oranges on sunflower plants. After 20 hours' exposure, plant *c* (ex-



FIGURE 4.—Epinasty caused by emanations from sound and decaying Valencia oranges and from pure cultures of *Penicillium digitatum*. *A* and *B*, Sunflower plants. *A*, Effect of Valencia oranges: *a*, Control; *b*, exposed to sound oranges; *c*, exposed to oranges showing stem-end decay. *B*, Effect of *Penicillium digitatum*: *a*, Control; *b*, exposed to *P. digitatum*. *C*, Tomato plants: *a*, Control; *b*, exposed to *P. digitatum*.

posed to oranges showing stem-end rot) showed more pronounced epinasty than plant *b* (with sound oranges). Plant *a* is the control.

Evidence was obtained showing that pure cultures of *Penicillium digitatum* are capable of producing epinasty in potato, tomato, and sunflower plants. In figure 4, *B*, is shown the effect of emanations from this fungus on sunflower plants. Plant *a* is the control, and plant *b* is the treated plant. In figure 4, *C*, will be found similar results with tomato plants. Similar results were obtained repeatedly with all test plants used.

Results with pure cultures of *Diplodia natalensis*, *Diaporthe citri*, and *Alternaria citri* were either negative or inconclusive.

DISCUSSION

In view of the large number of fruits that have been shown to evolve ethylene, it is not surprising that citrus fruits may now be added to the list. From observation of the degreening of oranges, lemons, and grapefruit with ethylene, the writers have long believed that ethylene might be a natural byproduct of metabolism in citrus fruits. Earlier investigators had suggested that ethylene degreening of citrus fruits merely accomplished what was brought about by nature in a somewhat longer time. The results obtained in the present investigation tend to verify this statement.

The question of the effect of essential oils naturally arises because of the large quantities present in this type of fruit. Tests made with cold-pressed orange oil indicated that these substances were not responsible for the epinastic response of the test plants. Similar results have been reported by Denny (3) and Elmer (4).

The apparent increased evolution of ethylene by decaying fruit is a little difficult to explain. It is generally conceded that maximum evolution of ethylene by apple fruits occurs during the "climacteric." This stage in the life process of the apple is coincident with the maximum rate of production of carbon dioxide and corresponds to the period when the apple is fully ripe. It is also known that the rate of carbon dioxide production rises again when decay or break-down begins. It seems reasonable to suppose that ethylene production should increase again with the second peak in carbon dioxide production. A practical application is at once suggested. The presence of decaying oranges in a storage room may be instrumental in hastening senescence in other fruits in the same container or even in the same room.

The evolution of ethylene by fungi presents a relatively new field of investigation. Although *Penicillium digitatum* is the only organism of those studied that gave positive results, it is very probable that others may be capable of evolving ethylene. *Penicillium* is a fast-growing fungus and produces spores very readily. It is possible that during the formation of fruiting bodies a fungus, like an apple, may attain a climacteric.

A practical question is suggested here as in the case of the decayed fruit in storage. The storage life of surrounding fruits might be affected by the ethylene evolved by fungi. In view of the fact that in these experiments a relatively small quantity of the fungus produced the same effect as 24 oranges, the significance of decaying fruit in stored lots may be even greater than has been previously suspected.

SUMMARY

The production of epinasty in test plants, such as the potato or tomato, is generally conceded to indicate the presence of ethylene in the emanations of plant tissues.

Citrus fruits were tested for ethylene evolution, by using this biological method, and positive results were obtained with oranges, tangerines, limes, lemons, and grapefruit.

Oranges and grapefruit inoculated with *Penicillium digitatum* and oranges showing stem-end decay produced epinasty in the test plants sooner than did normal fruit under the same conditions.

Positive results were obtained also when pure cultures of *Penicillium digitatum* were tested for evolution of ethylene.

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A NEW VIRUS DISEASE OF SNAP BEANS¹

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INTRODUCTION

In June 1938, while inspecting a field planting of varieties and hybrid progenies of snap beans (*Phaseolus vulgaris* L.), the writer discovered a very destructive and, apparently, undescribed disease (4).³ During the growing season of 1939 the disease appeared again and was even more destructive than in 1938. This paper presents some preliminary results of a study of the symptomatology, etiology, transmission, and host range of the disease. Since the disease has thus far not been given a common name, the writer proposes the designation "black root."

REVIEW OF LITERATURE

Several virus diseases of snap beans have been described and their etiology listed as *Phaseolus* virus 1, 2, and 3 (1, 3, 5, 6); yet other viruses are known which, although they are classified in other taxonomic categories, produce disease on snap beans. It is this last-mentioned group of viruses that produce symptoms on snap beans most nearly approaching those of black root.

Smith (6), p. 333, states that *Datura* virus 1 causes a systemic infection in snap beans accompanied or followed by a vascular necrosis in the stem, and the entire plant is killed. He makes no mention of external symptoms or of vascular necrosis of the roots, pods, and leaves.

Nicotiana virus 12 is reported (6, 8) to cause necrotic spots on the leaves and necrosis of the veins on snap beans. In addition, systemic infections occur which are characterized by sunken lesions on the stems and vascular necrosis of the stem and roots. No mention is made of symptoms on the pods.

Whipple and Walker found that *Cucumis* virus 408 (7) produces disease symptoms on 21 species in 5 families, including 4 varieties of *Pisum sativum* and 39 varieties of *Phaseolus vulgaris*. Among the latter are varieties resistant to *Phaseolus* virus 1. Mild streaking of the stem and mottling of the leaves of young plants were produced on peas; while distinct mottling, leaf distortion, and stunting occurred on bean and, with one substrain, a severe streak. No mention is made of vascular necrosis of the roots, pods, and leaves.⁴

¹ Received for publication August 11, 1939. Paper No. 64, Journal Series, Georgia Experiment Station.

² The writer wishes to acknowledge the suggestions given by Drs. B. B. Higgins and J. L. Weimer. He also wishes to thank M. M. Murphy, Jr., for making the photographs.

³ Italic numbers in parentheses refer to Literature Cited, p. 288.

⁴ Through correspondence with Whipple, the writer has learned that although *Cucumis* virus 408 produces some necrosis of the root and lower hypocotyl on several hosts, necrosis is not regarded as a descriptive symptom.

DESCRIPTION OF THE DISEASE

In the field, the first noticeable symptom of the disease is an incipient wilting of the leaflets, usually near the apex of the plant, during blossoming or early podding time. This symptom is too easily overlooked, however, to be of much practical importance. The first symptoms of diagnostic value appear soon after the incipient wilting and consist of a yellowing of the lower leaves followed or often accompanied by a permanent wilting of a part or all of the plant (fig. 1).



FIGURE 1.—Photograph of a diseased bean plant in the field showing early phase of wilting. Note the abnormally dark-colored sutures of the pods in the foreground.

Severe chlorosis may precede or accompany wilting, apparently dependent on or coincident with the degree of vascular involvement.

Closer inspection shows a brownish-red to purplish-black streak or streaks running lengthwise of the stem above the cotyledonary node (fig. 2, *B, a*).

When the epidermis and outer cortex are peeled back, or the stem is cut in cross section (fig. 2, *B, b*, and *C*), the streaks can be seen as an intense, dark discoloration of the area between the inner cortex and outer xylem. Strictly speaking, the inner phloem and outer xylem are most intensely discolored during the early phases of the disease, but later the discolorations may extend deeper into the xylem and even to the pith by way of the xylem rays. The external appearance of the streaks is due to the masking effect of the chlorenchyma of the cortex rather than to a surface lesion.

Well-defined external streaks are also evident on the lower hypocotyl, and, in cross section, the vascular elements are intensely discolored in this area (fig. 2, *C*). The writer has never observed any

external indication of a water-soaked condition along the streaks. Sometimes the streaks are confined to one side of the stem and the organs on the affected side die. Cross sections of such stems show the vascular discoloration to be confined to the vascular elements in one side of the stem (fig. 2, *B, b*). Often the discoloration may cross over to the opposite side of the stem at a node, and the plant parts free from discoloration may continue to live, at least for a time. Under such conditions of limited vascular involvement, certain plants may survive long enough to mature viable seed, a point which will be discussed later. Pole varieties often exhibit considerable discolora-

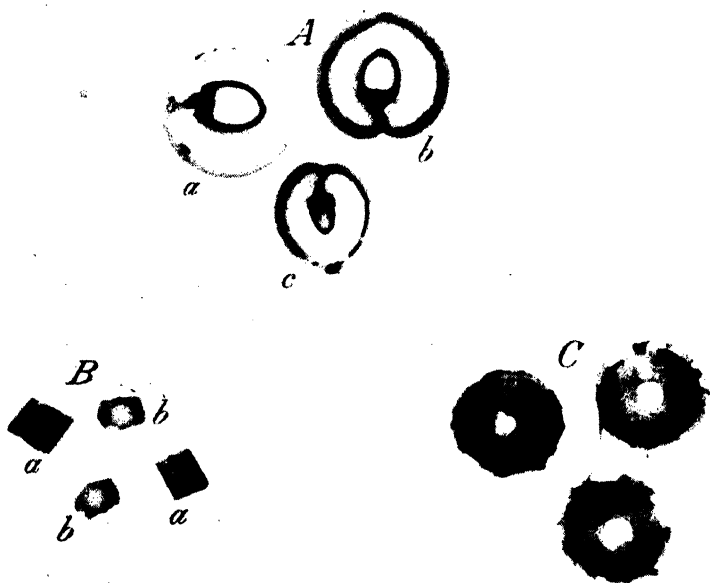


FIGURE 2.—Sections, unstained, of disease plant parts: *A*, Cross sections of diseased pods in the "snap bean" stage; *a*, Limited amount of vascular discoloration; *b*, and *c*, severe vascular involvement and mode of entrance into the seed. *B*, Plane and cross sections of diseased stems; *a*, Plane, surface view of streaks along the stem; *b*, cross sections showing fairly limited vascular involvement. *C*, Cross sections of diseased (lower) hypocotyls, showing intense vascular discoloration, inner bark, and outer xylem, as well as varying degrees of vascular flecking.

tion of the vascular elements of the median axis of the plant, but the disease is apparently confined mostly to this portion. This condition results in rapid killing of the terminal "runner," while other parts of the plant are only slightly affected, even over a considerable period of time.

Under certain other conditions of generalized infection, entire plants may wilt rapidly, apparently out of proportion to the amount of vascular discoloration usually associated with the death of plants

or of plant parts. This observation naturally raises the question as to how closely infection and discoloration may be correlated.

The diseased parts of the plant, except for the leaves and young pods, always remain firm after death, but are in a rather desiccated condition.

On the older leaves, aside from early symptoms of incipient wilting, there usually appears more or less severe chlorosis (fig. 3, *C*). The color becomes pale at first, but rapidly fades to yellow, with some green remaining in and bordering the veins. These symptoms are accompanied by a definite flagging of young leaflets, followed by a

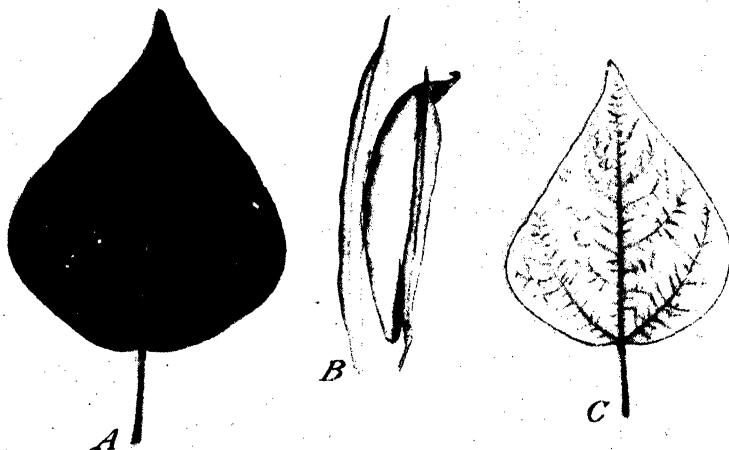


FIGURE 3.—A, Normal leaflet from a healthy plant; B, diseased pods showing the slightly darkened sutures; C, chlorotic leaflet from a diseased plant.

permanent wilt which usually begins at the tip or on one side of the leaflet. Under certain conditions of apparently slow death, the leaflets fade to yellow and finally to a parched brown color and fall from the plant. Under other conditions of rapid death, the leaflets wilt and dry out and curl rapidly without undergoing chlorosis. Sometimes the pulvini appear translucent but never water-soaked. The petioles do not flag but characteristically remain rigidly upright. Vascular discoloration is evident in the petioles and under certain conditions extends visibly into the veins and veinlets of the leaflets.

A careful inspection of leaflets from diseased plants, particularly when the light is favorable, shows a distinct mosaic pattern. The symptoms are not so pronounced as in the case of true bean mosaic. There is no puckering, curling, or other distortion of the lamina; only a well-defined mosaic pattern of pale and dark-green areas, more or less in a checkerboard pattern. On standing in water, or when wet by rain or heavy dew, the light areas become water-soaked in appearance.

The streaks also enter the peduncles and pods, but on the pods external symptoms appear only along one or both sutures; never

elsewhere. Here, the chlorenchyma again masks the color, causing it to appear as a slight anthocyanescence (fig. 3, *B*). When the chlorenchyma is removed, the vascular elements of the sutures and the lateral pod walls are intensely and more or less generally discolored, depending on the degree of infection. Cross sections of the diseased pods show a variable distribution of vascular discoloration (fig. 2, *A*, *a*, *b*, *c*). When young pods become infected they shrivel considerably, finally become extremely hard and brittle, but do not rot. In cross section they appear intensely discolored throughout. Somewhat older pods, in the snap-bean stage, generally show a more open distribution of vascular discoloration, and when severely infected the "pulp" sometimes presents an inky appearance as a result of its translucence and the reflection of the vascular discoloration. Young seed in severely infected pods are blasted. They often present a slimy appearance but do not rot.

Vascular discolorations can also be traced into the seed by way of the funiculus (fig. 2 *A*, *b*, *c*). If the plant and pods are not too severely infected, viable seed are often matured. White seed coats show external yellowish discolorations while self-colored seed coats, particularly black ones, become severely flecked with rust or deep buff-colored areas, i. e., color breaks.

Externally, diseased roots show no other evidence of infection than a generalized darker color, although in severe cases the taproot may appear almost black. Here, as on the above-ground parts of the plant, not all members of the root system are affected. The taproot, however, never escapes. In cross sections of diseased roots vascular discoloration is even more striking than in the above-ground parts, owing, perhaps, to the fact that there are relatively more vascular elements in the somewhat older roots and xylem discoloration is more extensive. Roots that have apparently been dead for some time do not rot.

PATHOLOGICAL ANATOMY

As stated above, the first visible evidence of vascular involvement appears as a brown to almost black discoloration of the tissues lying between the inner cortex and the outer xylem. The cambial area is an exception, at least for a considerable period after the other elements are quite discolored. In the upper stem, the discoloration does not usually extend very deep into the xylem, while in the lower hypocotyl and taproot extensive flecking may finally extend deep into the wood and even follow the xylem parenchyma almost into the center of the axis. Thin cross and longitudinal sections of diseased tissues show no evidence of a morphological break-down of any tissues, but gummy substances are present, as well as tyloses, particularly in the xylem vessels. The discoloration appears to be located principally in the walls of the affected vascular elements. Where gum and tyloses are present they are usually discolored.

From the evidence in hand, it appears that the degree and rapidity of wilting of diseased plants, while contingent on the severity of infection, is primarily correlated with the extensiveness of gum and tyloses formation. The root system is mechanically blocked and thus rendered physiologically inadequate to supply the plants with water at a time when this substance is in great demand. To what extent the virus alone is able to accomplish this result and how far

micro-organisms and other physiological factors may be concerned in the process, are yet to be determined. It is a matter of common observation that diseased plants will wilt rapidly in the presence of abundant water.

ETIOLOGY

Considerable difficulty was encountered in the determination of the etiology of this disease. From the symptoms, development of wilting, field distribution, spread, etc., it appeared logical to suspect a fungus or bacterial pathogen. Accordingly, numerous isolation experiments were conducted in which various techniques, parts of the plant, degree of infection, early and late symptoms, media, etc., were used. Although the sum total of organisms isolated was large, no one organism ever appeared consistently either in series or from time to time. Among the various organisms isolated were: Bacteria, *Fusarium* spp., *Neocosmospora* sp., *Aspergillus* spp., *Penicillium* spp., *Sclerotium rolfsii* Sacc., *Rhizoctonia solani* Kühn, *Macrophomina phaseoli* (Maubl.) Ashby, and *Pythium butleri* Subr. Representatives of all isolates were inoculated separately into the roots and hypocotyls of young bean plants, and were also used on appropriate media (mostly sterilized oats) to infest sterile soil into which beans were subsequently planted. All the work was carried out under controlled conditions in the greenhouse. Of the organisms used, those pathogenic to beans always produced characteristic symptoms, but in no case did the resulting disease display the symptoms of the black root disease. As a final effort to find a pathogenic organism, certain bean root nodules which appeared somewhat atypical were crushed in sterile water and injected into the hypocotyls of healthy plants. This inoculum likewise failed to produce symptoms of the black root disease.

Because of the peculiar distribution of organisms in the plant parts used in the isolation work, and their sporadic appearance on all the plant parts (except from the pods and from the young seed removed aseptically from fresh diseased pods, which consistently remained sterile in the isolation work), it was thought advisable to try some gross inoculation work. Accordingly, soil was potted directly from beneath dead and dying bean plants in the field, and bean seed was planted in it immediately. This soil contained large quantities of broken, diseased roots, nodules, etc. In conjunction with this experiment, composted, sterile soil was thoroughly mixed with freshly cut, diseased plant parts, including pods, stems, leaves, and roots. Bean seeds were planted in this soil immediately after mixing. All pots were covered with insect cages before or just after the seedlings began to emerge, and the pots were subsequently watered from the bottom so that the cages would not need to be opened. In no instance did any of the plants develop disease symptoms like those of the black root disease. In fact, the only disease that did develop was due to *Pythium butleri*.

TRANSMISSION

The nature and the number of the inoculation trials with micro-organisms, all of which failed to produce the disease under the conditions of the experiments, led the writer to the conclusion that the disease is due to a virus. The following experiments were accordingly conducted to determine the mode of transmission.

Several sweepings of diseased and adjacent bean plants were made in the field, and from 3 to 12 insects of 8 different genera were trapped. These insects were brought immediately into the laboratory, separated, and caged by species on separate healthy young bean plants growing in sterilized composted soil. The insects¹ so tested as possible vectors were: Spotted cucumber beetle (*Diabrotica duodecimpunctata* (Fab.)); bean leaf beetle (*Cerotoma trifurcata* (Forst.)); three-cornered alfalfa hopper (*Stictocephala festina* (Say)); Rapid plant bug (*Adelphocoris rapidus* (Say)); tarnished plant bug (*Lygus pratensis* (L.)); a broad-headed bug (*Coriscus pilosus* (H.-S.)); a member of the chinch bug family (*Geocoris punctipes* (Say)); and a small brown leafhopper, not determined.

With the exception of one doubtful case, none of the insects transmitted the disease under the conditions of the trials. In this case, out of several plants infested with the tarnished plant bug, a single plant developed symptoms characteristic of the disease. Although this single case may be significant, the presence of the disease might be explained on the basis of an infected seed. At least the experiment must be repeated with large numbers of both plants and insects before it can be stated definitely that the tarnished plant bug is a vector of the disease.

In conjunction with the insect studies, various types of grafts were tried in an effort to transmit the disease, but owing to the difference in ages of the scion and host tissues, or to technique, no union of tissues resulted. These unsuccessful grafts likewise failed to transmit the disease. In certain cases, when fresh diseased tissue was inserted under the bark and into wounds in the hypocotyl of healthy young plants, discoloration of the vascular tissues was evident for a few centimeters beyond the wound after a week; but the experiment had to be discontinued before completion because of a serious infestation of white fly (*Aleurodes* sp.).

The only successful artificial transmission of the disease was accomplished by hypodermic injections of juice from fresh, diseased pods. The pods in the snap-bean stage were brought to the laboratory and immediately crushed in a sterile porcelain mortar with a porcelain pestle. The juice, undiluted, was filtered through several thicknesses of cheesecloth to remove the plant parts, poured into a glass hypodermic syringe, and injected immediately into the hypocotyls and stems of young, healthy, caged bean plants in the greenhouse.

When injected on June 26, 1939, the bean plants were just showing the first compound leaf. On July 10, flower buds were beginning to form and the vascular system of the plants showed faint discolorations which extended 2 to 3 inches above and below the lowest and the highest of the needle punctures. On July 17, certain inoculated plants were showing fairly intense vascular discolorations up through the second compound leaf and faint vascular discolorations almost to the tip of the plant. Meanwhile, vascular discolorations had progressed downward to the base of the taproot. Although at this time the plants were not permanently wilted, certain leaves showed an incipient wilt, and the lower leaves exhibited symptoms typical of those of diseased plants in the field. A week later, the plants began to die. The leaves wilted and dried out in typical fashion, but for

¹ The writer is indebted to T. L. Bissell, station entomologist, for the identification of the insects.

some reason as yet unknown to the writer, vascular discoloration never reached the intensity of typical field specimens. Greenhouse conditions with the attendant etiolation of the test plants was no doubt a considerable factor in this discrepancy.

Attempted isolations from these inoculated plants showed no more evidence of bacterial or fungus pathogens than did diseased field plants. This phase of the work is being continued, as are also certain phases of the studies on pathological anatomy, in the hope that more information may be obtained on these two critical phases of the disease.

As regards natural transmission of the disease, the writer conducted an experiment the results of which appear highly significant.

On July 11, 1938, 25 seeds, which proved to be viable, were taken from diseased pods of a hybrid pole variety and planted in sterile soil in pots in the greenhouse. Shortly after the seedlings began to emerge, the pots were removed out of doors on account of the high temperature in the greenhouse. On September 2 reddish-brown streaks had appeared near the nodes on about 10 percent of the plants, and when the cortex was peeled back vascular discolorations were found which were identical with those observed on diseased plants in the field. The leaves arising from those areas showing streaks wilted and died. At the time of the last observation before death of the plants, they had not wilted permanently. Other work demanded the writer's attention at this time so that the course of the disease could not be followed in detail. However, the plants were seen again at a later date. At that time they were dead, or very nearly so, and in this condition appeared identical with diseased field plants at a comparable stage. The pods likewise showed typical symptoms, and a few seeds were saved.

Seed-transmission studies are being continued. Seeds are being used from the plants mentioned above in an attempt to determine, relatively, how many generations the virus can be transmitted through the seed.

HOST RANGE

Thus far, because of the difficulty of transmission and the long incubation period of the pathogen, the host range has been determined from observations on natural infections only.

The disease was first seen in the writer's breeding plots on the third generation progeny of Wisconsin Refugee \times Michella bean. It was next observed within a few days in a variety trial at Tifton, Ga.; this time on a commercial variety (Black Valentine pole). The seed of this variety had been secured the previous season from a commercial source. Subsequently, the disease was observed on practically all the commercial varieties in the writer's variety trials and on numerous segregating progenies. Specific notes have been taken on the course of the disease on Black Valentine pole, Black Valentine (bunch), Wisconsin Refugee, and White Kentucky Wonder (U. S. No. 3).

In addition to these snap-bean varieties, the writer has also found the disease on several naturally infected dwarf lima beans. In this case the seed was two seasons removed from the breeder. Unfortunately, the writer did not see the planting the previous year so that he is unable to state whether the disease was present on the plants the first year from the breeder.

DISCUSSION

Of the virus diseases of snap beans that have been reported in the literature, those caused by *Datura* virus 1, *Nicotiana* virus 12, and *Cucumis* virus 408 (substrain) perhaps most closely approximate the disease herein described. Whether any relationship exists between any of these viruses and that of black root is not known. The literature on *Datura* virus 1 on beans is very meager and the symptoms have not been described in sufficient detail to enable one to make an accurate comparison.

The symptoms produced by *Nicotiana* virus 12 on snap beans, as they have been described differ in several important respects from the symptoms of the black root disease as they have been observed by the writer. For example, the writer has never observed necrotic areas on the leaflets or sunken necrotic lesions on the stems in plants showing typical symptoms of black root; yet these appear to be cardinal symptoms of the disease caused by *Nicotiana* virus 12. Moreover, vascular discoloration of the pod, a very prominent symptom of black root, has not been described in connection with the former disease.

Although *Cucumis* virus 408 (substrain) produces some vascular discoloration of the root and lower hypocotyl, this symptom is apparently not prominent enough to be regarded as a descriptive symptom of the disease; nor does it appear to extend throughout the vascular system of the plant as in the case of black root.

Even though none of the diseases caused by viruses in other taxonomic categories produce symptoms on the snap bean identical with those of black root, several produce symptoms that are very similar. It is, therefore, evident that much cross-inoculation work must be done in order to determine whether or not any actual relationship exists between the viruses of one or more of these diseases and that of black root.

A very striking feature of black root, as previously mentioned, is the correlation between the stage of maturity of the host and the destructiveness of the disease. The writer has never found typical symptoms of black root in the field earlier than the blossom or young podding stage of the host, although he has searched diligently through thousands of individual plants. It is not reasonable to suppose that the plants are susceptible only at this stage of their development. Numerous field observations, inoculation trials, and the instance cited of the apparent seed-borne nature of the pathogen indicate an extended incubation period.

Whether the entire period intervening between inoculation and the time at which typical disease symptoms are evident may properly be termed an incubation period in the case of a virus disease is open to question. Certainly no other virus disease of snap beans, so far as the writer knows, has been reported as having such an extended incubation period.

It is, of course, entirely conceivable that the symptoms exhibited by wilting and dying plants may in a sense be secondary manifestations of physicochemical changes only indirectly related to those which we visualize as virus activity. A more definite statement of this viewpoint must await the results of later research on certain details of the problem now under consideration.

Whether the symptoms expressed by the writer in terms of "vascular discoloration" are in the same category as the symptoms on the bean

and on other plants affected by viruses and by various virologists termed "necroses," may, perhaps be a matter of interpretation. Insofar as the studies on the pathological anatomy of black root have extended, there is no evidence of tissue break-down or collapse, the usual physical signs of death of tissues. As stated previously, most of the discoloration is residual in the walls of the vascular elements; although in instances of abundant tyloses or gum formation, the cell contents are discolored. It is hoped that a more extensive study of the pathological anatomy of the disease will clarify the situation.

SUMMARY

What appears to be a new disease of snap beans in the vicinity of Experiment, Ga., is described.

The symptoms consist at first of an incipient wilt of a part of the plant, followed by permanent general wilt of all the leaves and death; although under certain conditions parts of the plant may continue to live for a short time. Accompanying these symptoms is an extensive discoloration in the vascular system of the root, stem, leaves, and pods. The lower leaves exhibit a faint but distinct checkerboard pattern of light and dark areas.

The disease has been transmitted by hypodermic injections of fresh juice from diseased pods in the snap bean stage and through viable seed from diseased pods.

Thus far, experiments with insect transmission have given negative results except possibly in one instance in which the tarnished plant bug (*Lygus pratensis*) was used.

The host range as thus far determined includes several varieties of bunch and pole beans, as well as a variety of lima beans.

Work is being continued on certain phases of pathological anatomy, transmission, virus characteristics, and host range.

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JOURNAL OF AGRICULTURAL RESEARCH

VOL. 60

WASHINGTON, D. C., MARCH 1, 1940

No. 5

INFLUENCE OF UNRESTRICTED GRAZING ON NORTHERN SALT DESERT PLANT ASSOCIATIONS IN WESTERN UTAH¹

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AN INTENSIVE STUDY OF RANGE DETERIORATION

The northern salt desert country of western Utah, important as winter range for livestock grazing, is at present characterized by heavy injury to the plant cover, resulting in reduced forage production and a serious replacement of the most valuable forage species by plants largely unpalatable to livestock. This condition was revealed by the results of a recent range survey of the Intermountain Region³ by the Intermountain Forest and Range Experiment Station. This survey gave unmistakable evidence of considerable deterioration of plant cover and excessive loss of forage values, and indicated that no small part of the deterioration was to be found on the winter ranges.

In view of these findings, it seemed desirable to make an intensive study to ascertain the nature and causes of this condition as a basis for future management of the range. This paper deals with the data obtained in an intensive study of the plant associations on desert range near Milford, Utah (fig. 1), undertaken in 1933 and 1934, and checked in the field in 1935 and 1936. The associations studied are described in the light of their soil and climatic environments, and evidence is set forth relating their condition to the effects of unrestricted grazing.

The evidence of the data presented is substantiated by the almost unanimous assertions of explorers, such as Carte de Escalante (1, pp. 125-242),⁴ and of old-time residents of this region, all indicating that in the brief span of one generation an unmistakable deterioration has taken place in the quality of forage as well as in the quantity produced. Many stockmen admit forage deterioration on desert ranges, but part of them, at least, hold drought to be the principal cause. This opinion is not borne out by actual drought data taken on desert areas of this region comparable with those on which the present study was pursued. A brief review of these data makes this clear.

Precipitation records for the region from 1897 to 1936 are summarized in figure 2, C, combining the records of two stations, Modena and Frisco. Modena lies 35 miles to the south of Pine and Wah Wah Valleys, in which the study was made, and Frisco is a few miles east of Wah Wah Valley. The temperature-precipitation graphs shown for these two stations (fig. 2, A and B) give evidence of general agreement. In later years, records at three other stations, Milford and Black Rock,

¹ Received for publication October 25, 1939.

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³ The States included in the Intermountain Region are Utah, Nevada, southern Idaho, and southwestern Wyoming.

⁴ Italic numbers in parentheses refer to Literature Cited, p. 316.

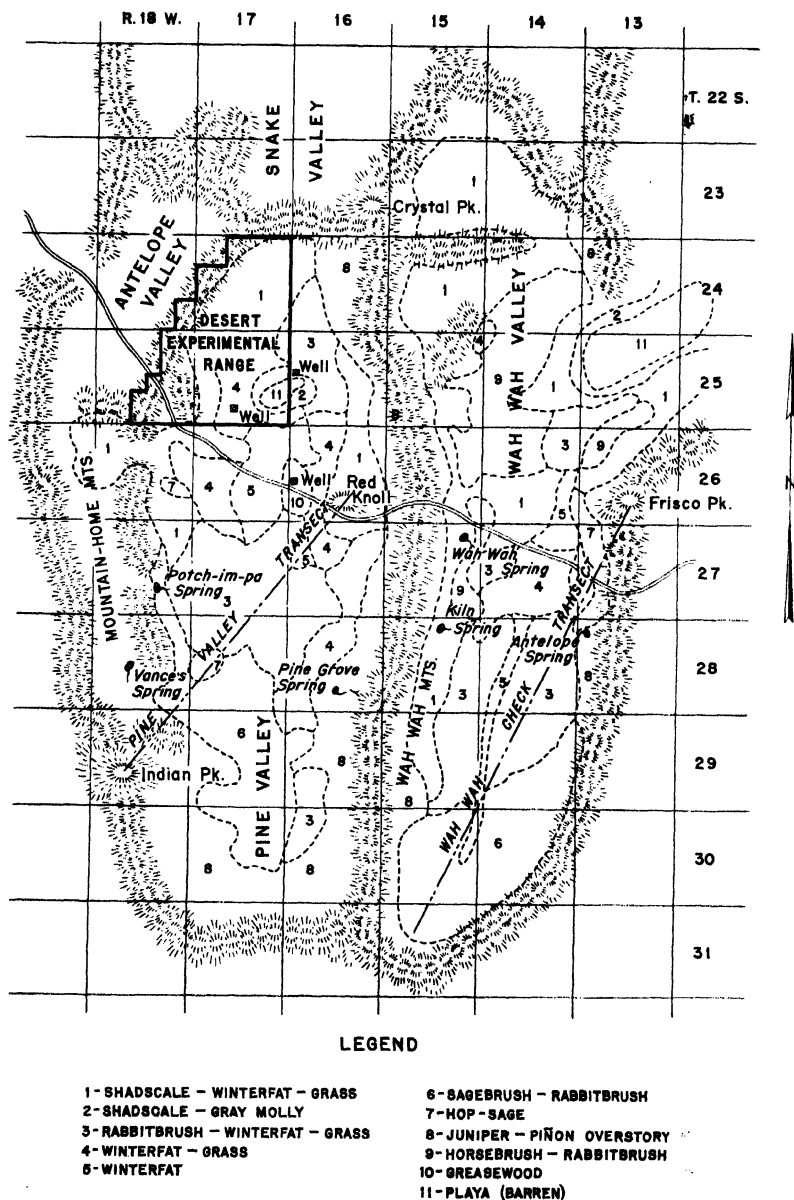


FIGURE 1.—Map of Pine and Wah Wah Valleys and surrounding territory near Milford, Utah, where the present study was pursued, showing forage types and transects run.

east and northeast of Frisco, and Garrison, to the northwest of Antelope Valley, have been in general agreement with those of Modena.

A marked significance in the 1897-1936 precipitation curve in the study of the desert plant associations is in the two drought periods,

1897-1904 and 1928-35. Of these, there seems little question that the former was as long as the other and the more severe. It does not, however, stand out in the memory of ranchers of that day as a period of forage impoverishment. Supplemental feeding of range livestock was unknown at that time; yet fat cattle were obtainable at any season of the year. Fat sheep were marketed on the desert range in midwinter during the decade 1895-1904, according to the testimony of resident stockmen. Such an achievement would have been utterly impossible in the recent drought period, and this very impossibility is eloquent of a radical change in the forage values of the range that is not wholly attributable to drought. The evidence is that in the early days the damaging effect of acute shortage of moisture in drought years was off-

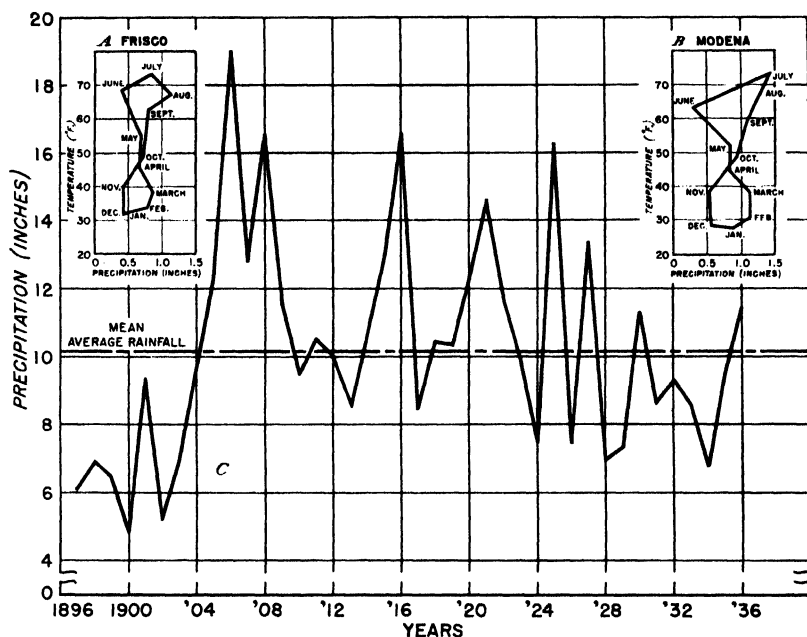


FIGURE 2.—Annual fluctuations in precipitation (C) in Pine and Wah Wah Valleys, 1897-1936; derived from records taken at Frisco (1897-1900) and Modena (1901-36). A and B are temperature-precipitation graphs of the two stations, averaging monthly mean temperatures and total monthly precipitation for the years of record.

set by moderate utilization, which permitted restoration of the normal growth and nutritional function of the range plants.

Drought is not new to the desert valleys of western Utah, but the present disastrous forage impoverishment is of comparatively recent origin. Since drought alone cannot account for the present deterioration in plant communities, nor explain the alarming plant retrogression, logic obviously points to another contributing cause sufficient to upset the vegetational balance, precipitate the downward trend in forage values, and in the two valleys in which the present study was made—subject to the same climatic vicissitudes but to widely different grazing use—to bring about the present great differences in plant mortality and forage volume.

How considerable these differences are may be realized from conditions in these valleys at the end of the recent drought period. The growth increment of all shrubs and grasses in both valleys was negligible in 1934, and in the normal growing season of that year the desert was almost destitute of green. Even the hardiest shrubs lost their leaves in the scorching summer days. Grass nibbled to the earth during the winter of 1933-34, in June, July, and August resembled stubble on badly abused pastures. Soil of thousands of acres of desert land, nearly barren of vegetation, seemed to be lifted away by the wind. Yet with good precipitation in 1935 and 1936, vegetation in the less heavily grazed valley made a comparatively abundant growth, whereas the heavily grazed plants in the other valley failed to respond except in small degree.

Basic facts brought out by this study, indicating the successional trends in desert vegetation, the severe injury from overgrazing to the more desirable forage plants, and their replacement by species of low palatability, should serve as guides to further research as well as in the subsequent administrative control required to bring about successful reproduction of the palatable shrub species and to restore the grasses.

DETAILS OF THE STUDY AREA

LOCATION OF STUDY

Pine and Wah Wah Valleys, which comprise a portion of the desert range near Milford, Utah, were selected for the study because they are characteristic of the drier parts of the Great Basin area. Furthermore, they are similar to each other in size, physiographic features, and plant associations; they support range types that are important throughout the desert area of the Great Basin; they represent distinctly different degrees of grazing use, Wah Wah Valley being the more severely grazed; and also their grazing history is better known than that of other desert areas.

Being nearer to the oldest settlements in the region, Wah Wah Valley has been grazed longer, i. e., for about 70 years as compared with 50 for Pine Valley. In the late eighties sheep were brought in great numbers to Wah Wah Valley and grazed there along with the cattle already present, thereby beginning a long period of double use. Cattlemen in Pine Valley succeeded for about 10 years after this in maintaining a dead-line against sheep. Cattle grazing in the late nineties was probably heavy in Pine Valley, but forage use did not become seriously destructive until about 1910 or possibly 1915. During the last 20 years, or thereabouts, largely through the development of watering places, forage utilization in Pine Valley has exceeded proper use in nearly, though not quite, the same degree that has been common in Wah Wah Valley over a period of 50 years. During the drought period of 1928-35, deterioration of the palatable forage plants became serious enough to be well marked in all parts of Pine Valley.

The climate of this locality is essentially typical of the drier parts of the northern shrub desert. The precipitation of less than 10 inches annually is generally distributed evenly throughout the year, although great fluctuations in the annual and monthly precipitation are characteristic. Potential evaporation, as in all deserts, is high—greater than 60 inches annually, so far as can be judged by records furnished

by the Weather Bureau for Lehi, Utah, situated near the desert edge. The diurnal range of temperatures is great at all seasons, extremes varying from a maximum of more than 100° F. in summer to a minimum of -35° in winter. The sky is so clear and the humidity so low that even in July the nights are cool. The frostless season of 4 months extends from mid-May to mid-September. Winds are fairly frequent and of rather high velocity.

Major desert plant associations are characterized by four general features: (1) Preference for certain soil conditions both of physical nature and saline content; (2) a rather sharp delimitation of the associations; (3) the preponderance of one or two plant species; and (4) the general absence of annuals, except for early spring forms, which in years of ordinary moisture supply are found on the foothills. In contradiction to this generalization is Russian-thistle,⁵ an exotic annual that has recently invaded badly deteriorated areas throughout the region.

The position, extent, and number of the various plant associations of Pine and Wah Wah Valleys shown in figure 1 suggest a confusing complexity of vegetation, but there are only two major growth forms, the piñon-juniper of the desert mountain ranges and the short desert-shrub type of the valley plains. The specific associations of the latter present a variety of color and differ in many details, but in their general features they are remarkably uniform in their morphological adaptations to the vicissitudes of the desert environment.

COLLECTION OF DATA

Investigators in the field obtained data on soil, root penetration, and vegetative status of the plant associations. Data on plant associations covered extent and habitat, species composition, degree of injury by grazing use, and the present condition of important species as measured, singly or collectively, by general vigor, presence or absence of young plants, and replacement of palatable by less palatable forage species.

A range survey of the two valleys gave the total number of square miles occupied by each of the 10 plant associations (table 1). Each association, several of which cover from 100 to 600 acres, consists of a mixed plant population of 10 to 20 species of which 2 or 3 are dominant. Within each association are often found plant communities of $\frac{1}{4}$ to 5 or 6 acres in area (rarely 40 or 50 acres) occupied by one of the major species in nearly pure stand. Soil samples discussed later were taken in these plant communities; the influence of grazing, however, was studied by the status of individual forage plants or groups of plants, sometimes in one association and sometimes in several.

Besides the general survey, detailed studies were made by examining the vegetation along one transect in Pine Valley and another in Wah Wah Valley. In Pine Valley the transect extended a distance of 23 miles from the base of the Wah Wah Mountains in a southwesterly direction across the valley to the summit of Indian Peak (fig. 1). In all, 920 circular point-observation plots (7), each with an area of 200 square feet, were established along the transect at intervals of 132 feet. In Wah Wah Valley, along a similar transect, observations on 180 plots

⁵ A list of scientific and common names of the plants discussed in this paper is given on p. 315.

were made. From each plot, data were obtained on vegetation composition, density of cover, and plant mortality.

TABLE 1.—*Areas occupied by various desert plant associations in Pine and Wah Wah Valleys*

Plant association ¹	Pine Valley	Wah Wah Valley	Plant association ¹	Pine Valley	Wah Wah Valley
	<i>Square miles</i>	<i>Square miles</i>		<i>Square miles</i>	<i>Square miles</i>
Shadscale—winterfat ² -grass.....	115	186	Spiny hop-sage.....	5	11
Shadscale—gray molly.....	7	15	Horsebrush — small rabbit-brush.....		76
Small rabbitbrush — winterfat ² -grass.....	105	102	Greasewood.....	5	
Winterfat ² -grass.....	73	22	Juniper—pifion overstory.....	370	223
Winterfat ²	25	16	Playa (barren).....	3	27
Big sagebrush—small rabbit-brush.....	75	79	Total area.....	783	757

¹ For scientific names of plants see p. 315.

² Locally known as white sage.

A plant was considered to cover the ground completely in the rare instances when the ground could not be seen through its foliage by an observer standing directly over it. In most cases the value recorded was the fractional spread of the plant when so compressed as to make a complete cover. Thus, if a plant with a total natural spread of 1 square foot made a complete cover of only one-half square foot, it was given a ground-cover (density) value of one-half square foot. When the total ground-cover area for all plants of a given species within a plot totaled less than one-fourth square foot, the presence of the species was recorded merely as a trace. The total area of all vegetation on a 200-square-foot sample plot was determined by adding the individual figures. Density percent was obtained by dividing the total area in square feet by 2.

Mortality data were obtained by counting the number of living and dead plants of each major species on the plots.

In addition to the transect composed of circular plots, small "belt" transects were established near but not on the main transect in each plant association. Care was exercised to locate these at random. On the belt transects vascular ring counts were made of 100 shrubs of each major species in each association from all plants whose crown centers fell within the transect boundaries. Two men, equipped with hand lenses and trained to take the age data, made separate counts of the vascular rings for each stem and compared results. If, owing to the irregularity, incompleteness, or indistinctness of the vascular rings, large discrepancies occurred, recounts were made. In order to check the accuracy of the method, ring counts were made on stems taken from plants on railroad fills and in an abandoned cemetery, where the maximum possible age of the shrubs was known.

The type of root system, the horizontal and vertical spread of the roots, and the general relationship of root penetration to the soil-salt horizon were recorded for each major species. These data were obtained from excavations made in associations dominated by the species.

NATURE OF SOILS AND SOIL REGION

Soil samples were taken in several plant communities in the associations in Pine Valley with the exception of the juniper, which grows in very shallow soil. In all, 14 trenches were dug in the various communities, and profile maps of the soil horizons were drawn. Three to five soil samples were composited from the trenches at each of the following depths in centimeters: 0-15, 15-30, 30-60, and 60-90. Each sample was carefully analyzed for total salts, total organic matter, pH value, total nitrogen, moisture content, moisture equivalent, water-holding capacity, and the percentages of gravel, sand, silt, and clay (table 2).

TABLE 2.—*Soil data¹ from the major plant communities indicated, occurring in various associations crossed by the Pine Valley transect*

Plant community within associations	Depth of soil	Average salt content (bridge)	pH value	Total organic matter	Total nitrogen	Moisture equivalent	Water-holding capacity ²	Soil moisture ³	Gravel	Sifted sample		
										Sand	Silt	Clay
Grass (blue grama, galleta, sand dropseed).	C'm.	<i>P. p. m.</i>		<i>Pct.</i>	<i>Pct.</i>			<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>
	0-15	200	8	1.25	0.091	18.1	37	2.2	59	62	29	9
	15-30	210	8	1.29	.102	18.6	47	3.3	50	51	43	6
	30-60	895	8	1.21	.101	20.8	51	2.6	66	59	34	7
	60-90	1,160	8	1.47	.094	20.7	37	2.9	74	57	34	9
Shadscale	0-15	200	8	1.16	.082	17.9	35	1.5	48	64	27	9
	15-30	468	8	.85	.094	18.5	44	2.1	27	53	41	6
	30-60	1,065	8	1.14	.087	24.8	45	2.4	80	64	27	9
	60-90	5,065	8	.69	-----	24.6	54	2.1	72	68	30	2
Winterfat	0-15	290	8	1.29	.087	20.3	54	1.9	32	54	26	20
	15-30	290	8	.63	.058	22.1	46	3.1	1	46	40	14
	30-60	6,015	8	.54	.054	21.0	64	4.9	0	54	44	2
	60-90	3,742	8	.26	.050	13.6	38	4.3	71	72	28	0
Small rabbitbrush	0-15	342	7.5	.80	.058	14.5	30	1.8	23	74	15	11
	15-30	962	8	.56	.046	15.4	35	4.2	18	75	13	12
	30-60	1,358	8	.32	.045	14.2	44	6.7	15	73	14	13
	60-90	8,648	8	.28	.034	30.0	59	8.1	12	47	39	14
Spiny hop-sage, blue grama, galleta.	0-15	244	7.5	.67	.061	11.9	33	2.7	19	92	3	5
	15-30	582	8	.41	.043	12.6	38	4.8	4	89	5	6
	30-60	323	8	.58	.141	8.7	46	4.9	6	93	4	3
	60-90	684	8	.50	.038	11.7	37	5.2	6	93	4	3
Big sagebrush	0-15	200	7.5	1.03	.061	13.5	37	3.2	16	82	15	3
	15-30	302	7.5	.84	.060	15.1	43	4.5	8	78	16	6
	30-60	716	8	.72	.050	11.6	44	5.0	9	76	18	6
	60-90	1,080	8	.21	.027	-----	34	5.0	12	84	16	0

¹ Based on average of 3 or more samples.² Hilgard's method.³ July 1934.

The soils of the Pine Valley transect are characterized by five general features: (1) A predominantly sandy nature; (2) a low saline content in the first 30 cm. of soil, regardless of the plant communities; (3) a variable saline content below the 30-cm. level, closely correlated with the type of vegetation; (4) a hydrogen-ion concentration between pH 7.5 and pH 8; and (5) an extremely low content of organic matter and nitrogen.

Physical differences in the soils supporting the various plant communities are slight. The degree and direction of slope, the presence or absence of hardpans, and the variation in the salt content of the subsoil seem to play an important role in the extent and distribution of the desert vegetation. For example, spiny hop-sage

and big sagebrush grow on soils which, in the main, contain little salt (4, 6) to a depth of about 3 feet (90 cm.) or more. The subsoils are more porous, and their water-holding capacity is lower.

All soils in these valleys have a greater amount of gravel at the surface than immediately below; winterfat soils in some cases have 32 times as much. From the condition of vegetative cover in the



FIGURE 3.—Aspects of erosion in Wah Wah Valley: *A*, General view of a wind-eroded area in the winterfat association; *B*, pedestaled winterfat plants.

various associations it may be deduced that the surface accumulation is not so much an index of percent of gravel in the soil as of the deterioration of the vegetation, which when severe permits rapid wind erosion. The stifling clouds of dust that race over the desert valleys are fed from areas largely denuded of vegetation (fig. 3, *A*).

That a heavy soil movement is occurring on this area is borne out by several observed facts. The pedestaled nature of many desert plants, especially winterfat (fig. 3, *B*), shows that 1 to 6 inches of soil have been removed from below their crowns since their establishment. In

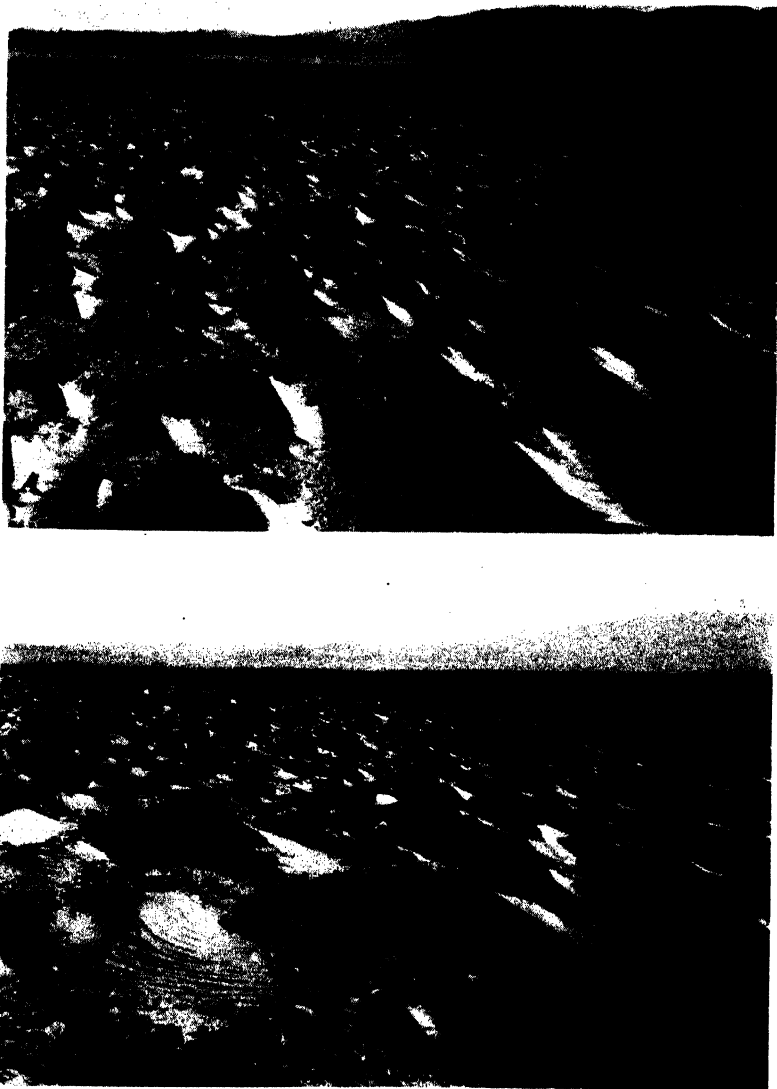


FIGURE 4.—*A* and *B*, Embryonic dunes in Wah Wah Valley, winterfat association.

such cases the height of crown above the soil is not an age factor, for many young plants protrude as much as old ones. Except near the relatively few natural dune areas, pedestaled plants occur only in associations obviously deteriorated through grazing. Many embryonic dunes (fig. 4, *A* and *B*) along highways and on the plant areas

under observation are known to be but a few years old; and a juniper forest on a presettlement dune area in the northeast corner of Pine Valley shows what appears to be a greatly accelerated recent and disastrous dune movement (fig. 5, *A*).



FIGURE 5.—Aspects of erosion in Pine Valley. *A*, Juniper trees buried by sand dune; *B*, an advancing dune in the juniper-piñon association.

Groves of dead junipers newly buried by sand in this old dune area stand as mute evidence of newly aroused activity of dune movement, and living young junipers scattered in this ghost forest, at various distances in the rear of an advancing dune (fig. 5, *B*), furnish evidence as to the average annual dune movement compiled in table 3.

TABLE 3.—Rate of dune movement in Pine Valley, as estimated from ages of living juniper trees, in rear of advancing dunes

Tree No.	Distance of tree from dune	Age of tree	Estimated yearly movement
	<i>Feet</i>	<i>Years</i>	<i>Feet</i>
1	608	25	24.32
2	830	31	26.77
3	881	38	23.18
4	807	40	20.18

ROOT PENETRATION OF PRINCIPAL DESERT PLANTS

The root penetration of desert plants, as shown by the nature and extent of the root systems, is influenced by two major factors, soil salinity and the nature of the plant.

The four grasses, blue grama, galleta, Indian ricegrass, and sand dropseed have root systems the large branches of which are entirely within a shallow soil layer no deeper than 30 to 45 cm. with a salt

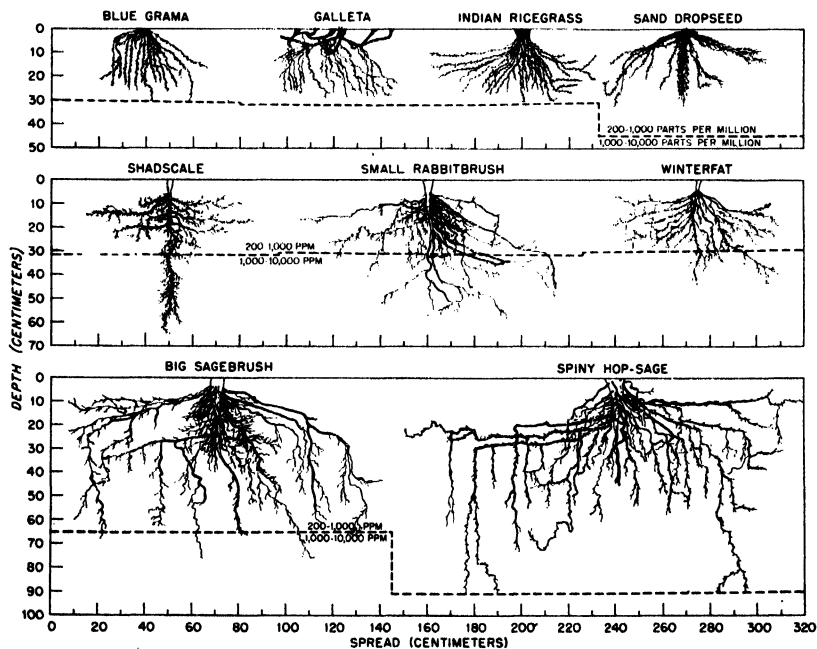


FIGURE 6.—Root penetration of nine principal desert plants, indicating their respective endurance of salt salinity. The heavy broken line marks zones of salt content—above, 200 to 1,000 parts per million; below, 1,000 to 10,000.

content of less than 1,000 p. p. m. (fig. 6). The roots are finely branched and occupy this surface layer rather fully. The roots of shadscale, small rabbitbrush, and winterfat, on the other hand, have some larger roots that penetrate into soil layers having a salt content in excess of 1,000 p. p. m., and also have a developed taproot system. Big sagebrush and spiny hop-sage send down roots to approximately 80 or 90 cm., but the soils supporting these two species are usually

porous and newly deposited alluvial material, frequently in front of a ravine. This is especially true of the hop-sage, which was always found near the base of an alluvial cone where storm floodwaters spread out and afforded irrigation. Although sagebrush often sent a few roots into a soil layer of greater salinity, in no case were hop-sage roots found growing in soil that had a salt content of more than 1,000 p. p. m.

Growing only in deep alluvial soils with the hardpan 3 to 5 feet below the surface, the roots of hop-sage and sagebrush offer less competition to grasses in the associations dominated by these two plants than is encountered from shrubs in other associations, such as shadscale, rabbitbrush, and winterfat. Since these last-named species usually grow on soils having a depth of only 12 to 24 inches above hardpan, most of their roots occupy the same soil layer as those of the grasses and therefore compete directly with them for moisture. Both historical and ecological evidence point clearly to the greater previous abundance of valuable grasses in the sagebrush and hop-sage associations.

EVIDENCE OF DETERIORATION IN PLANT ASSOCIATIONS

DESERT GRASSES

Grasses, which today are insignificant in most of the desert-shrub associations, were originally important members of practically all of them (2, 5, 6). Old residents maintain that desert vegetation formerly



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FIGURE 7.—A good stand of winterfat in a lightly grazed valley in Nevada.

consisted of mixed shrub-grass associations, except in the low, broad watercourses along the valley edges where winterfat (white sage) existed in almost pure stands. (Fig. 7.) They say also that when grazing began this species was the principal shrub of the valleys, and that big sagebrush and black sagebrush (black sage), with abundant grasses interspersed, dominated the foothills.

It is probable that grasses never existed on the desert in pure stands of any great area, but were always associated with shrubs. At present they are most abundant on the foothills in a narrow belt lying between the shadscale association in the valley and the juniper association at the higher elevations. Shadscale is now the most important shrub component of the grass communities. Grass species, in order of their abundance in the Pine Valley transect, are blue grama, sand dropseed, galleta, and Indian ricegrass (3). The first three of these grasses, usually combined but sometimes occurring separately, frequently form almost pure communities over small tracts of less than an acre within the associations. This probably is to be explained rather by priority of occurrence than by reaction to peculiar soil conditions. In all grass communities, the sites have a gentle slope with good drainage, but

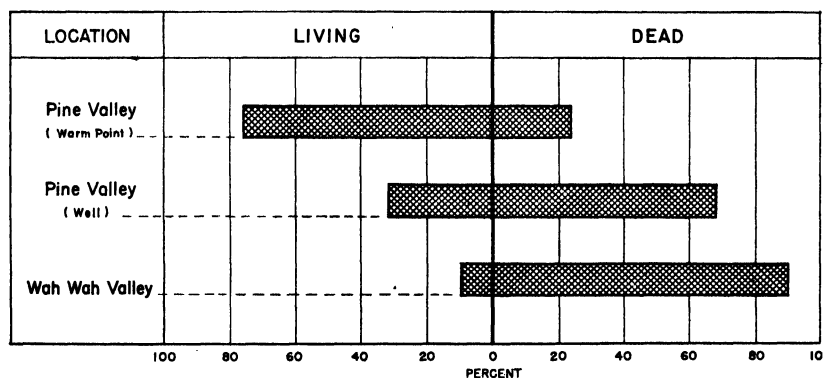


FIGURE 8.—Percent of Indian ricegrass plants alive and dead in three significant locations, the moderately used area of Warm Point, the now heavily used area adjacent to a well, and the long-used, severely grazed Wah Wah Valley.

in many cases the saline content of the soils below 30 cm. is high. At this depth, or slightly below it, a calcareous layer commonly exists, probably at the average depth of moisture penetration.

Indian ricegrass, probably the most conspicuous if not the most abundant grass on the desert in 1880, is now reduced to a very low density under the longer and more severe grazing practice in Wah Wah Valley. It readily adapts itself to many habitats, although it seems to be best adapted to sandy soils. It is significant that this most palatable of all desert grasses is threatened with almost complete extinction. That its disappearance on large areas of the desert is traceable directly to grazing is illustrated in figure 8. On three sites, comparable for Indian ricegrass production, except for their grazing history, widely different mortality conditions exist. At Warm Point, 10 miles from permanent water, there are four times as many living plants of this grass as in the vicinity of a well drilled 10 years ago, where water is always available to livestock. Near the well, more than half the still visible plants are dead as the result of overgrazing, trampling, and congestion of the livestock around water.

The total plant cover in those communities of Pine Valley (fig. 9) in which blue grama is dominant exceeds per unit area that in all other communities save sagebrush and juniper-piñon. The desert grasses, however, have a high mortality rate and are low in vigor from continued close cropping for the past 30-odd years.

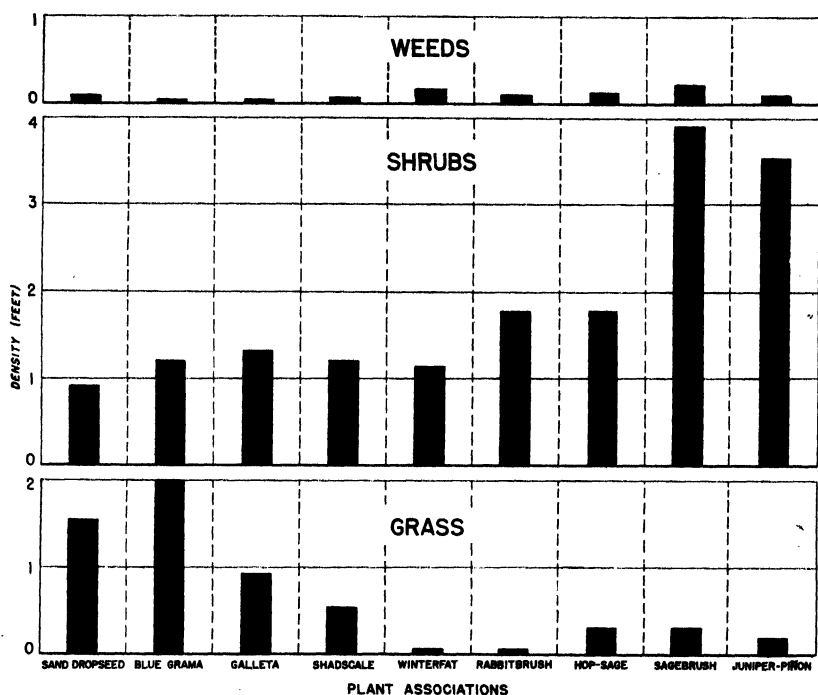


FIGURE 9.—Density of vegetation of shrubs, grasses, and weeds in the principal communities in Pine Valley, Utah.

Palatability of desert plants, besides indicating the relative extent to which they have been grazed in the past, affords a key to the interpretation of the successional trends of desert vegetation as revealed in this study. Under unrestricted grazing the more palatable plants such as Indian ricegrass, spiny hop-sage, and black sagebrush are seriously reduced, whereas the less palatable species such as small rabbitbrush, often untouched, have increased greatly in recent years. The data given in the following tabulation, from unpublished records of the Intermountain Forest and Range Experiment Station, represent the palatability of the current year's growth of the various species, or the percent that, on properly grazed ranges, is likely to be utilized by sheep and cattle during winter grazing:

	Palatability Percent
Grasses:	
Indian ricegrass.....	85
Sand dropseed.....	40
Galleta.....	60
Blue grama.....	40
Weeds:	
Russian-thistle.....	25
Shrubs:	
Spiny hop-sage.....	80
Black sagebrush.....	80
Winterfat.....	60
Shadscale.....	40
Small rabbitbrush.....	20
Big sagebrush.....	20

Desert grasses subjected to heavy grazing have reproduced very poorly during recent years. The age of grasses cannot be obtained with assurance since they do not have annual rings. It is thought, however, that with such grasses as blue grama, which in these high desert valleys increase the size of their clumps slowly, a rough separation can be made between old and young plants. Dropseed grass clumps also increase in size very slowly and somewhat regularly. Near absence of small plants in both valleys indicates an extremely low reproduction of these grass species during the last several years where the grazing was severe, whereas on a few lightly grazed tracts 3 or 4 miles from the transects young plants are fairly common.

On comparatively large areas in several associations of Wah Wah Valley galleta grass is now rather abundant, having occupied sites formerly producing abundant ricegrass. Numerous dead clumps of ricegrass are found on some sites now occupied by galleta, squirreltail, and Reverchon three-awn, galleta usually constituting most of the grass cover. This prevalence of galleta on severely grazed ranges is probably owing to its usual low height in the cool weather of fall, winter, and spring, which affords it some protection against full utilization, especially when a light fall of snow may cover it almost entirely. Since the open, irregular growth habits of galleta makes any estimate of its age highly uncertain, no measurements were attempted.

SHADSCALE

Shadscale dominates more square miles of valley desert than any other plant species and in Pine and Wah Wah Valleys occupies more than one-fourth of the total area below the juniper belt (fig. 10). Al-



FIGURE 10.—Shadscale association on Pine Valley transect, with Indian Peak in the distance.

kali-tolerant if not alkali-requiring (4, 6), it is found in two distinct soil habitats—the foothills where grass is an important member of the association, and the low, flat soils abutting the barren playa bottom where gray molly, a pronounced halophyte, is the chief commensal. On upland shadscale soils, hardpans rich in salt are found at 1 to 2

feet below the surface, whereas in the playa bottoms a high salt content is characteristic even of the surface soil.

Shadscale of the upland type has shown a mortality of approximately 30 percent during the drought period on both protected and unprotected areas, the heaviest losses occurring on its upper edge in the mixed shadscale-winterfat-grass association. However, this high mortality does not occur on the playa bottoms where the species is more vigorous. Since high mortality of upland shadscale followed the drought of 1931, it seems probable that this plant is better adapted to endure physiologically rather than physically dry soils, and that its preferred habitat is the heavier soil of the bottom lands with higher moisture-retaining properties.

Old residents of the desert maintain that on the upland habitats shadscale has increased both in acreage and in volume at the expense of winterfat and grass. Its competition with grass was perhaps greatest near the upper margins and with winterfat at the lower extensions of the association. No statistical information is available on its total volume on these upland habitats 50 years ago, but an analysis of the age⁶ and mortality data for the various components of the association indicates the extension of shadscale on both margins, as shown in table 4.

TABLE 4.—Percentage of shadscale and winterfat plants, in the upper and lower transition margins of a shadscale association, having 0-20 and more than 40 annual rings

Location and number of annual rings	Shadscale	Winterfat
Upper margin:	Percent	Percent
0-20	40	8
40	38	68
Lower margin:		
0-20	22	10
40	36	49

In view of the relatively low palatability of shadscale and the high palatability of winterfat, the data from their mixed association seem to warrant the following conclusions:

(1) On the average, shadscale is now suffering some loss of vigor from heavy grazing, as is shown by the fact that 50 plants (38 live ones) per plot have a ground cover of 3.2 percent in Pine Valley, in comparison with 44 plants (29 live ones) per plot and a ground cover of 1.6 percent in Wah Wah Valley. The high mortality of shadscale in all parts of the desert and equal occurrence of mortality on grazed and protected range, indicates low hardiness, against drought at least, on all sites.

(2) The ground cover of 10 winterfat plants in Pine Valley has been conservatively estimated at 1 square foot, whereas 59 plants per plot in Wah Wah Valley produced merely a trace, emphasizing the impoverishment of this species under prolonged heavy grazing. In Pine

⁶ It is recognized that, because winterfat, shadscale, and spiny hop-sage, which belong to the Chenopodiaceae, may under peculiar conditions lay down more than one vascular ring in a year, the total number of vascular rings may perhaps indicate only the approximate age, or somewhat more than the real age. In an abandoned desert cemetery, the oldest chenopods growing on graves dated 30 to 50 years ago failed to show any plant with a number of rings that exceeded the number of years since burial. Excess of ring counts over age in years does not therefore seem to be great for the period being considered. Since in nearly all cases, the ring counts are relative between the different species or between different samples of the same species during the same years, comparisons seem valid.

Valley 24 percent, and in Wah Wah Valley 55 percent, of winterfat plants in the shadscale-winterfat-grass association are dead (fig. 11) as the result of unregulated use.

(3) Similarity of the ring counts of the two shrub species in the well-established mixed associations indicates that they were associated during the early days of grazing on the desert. Thirty-six percent of the present shadscale plants, and 49 percent of the winterfat plants have more than 40 annual rings. That shadscale is invading winterfat communities is shown by the fact that 22 percent of the former in the transition zone has less than 21 rings, while only 10 percent of the latter falls in this class. Both the spines and the fact that the palatable leaf growth strips off leaving the unpalatable stems protect shadscale to some extent against excessive grazing.

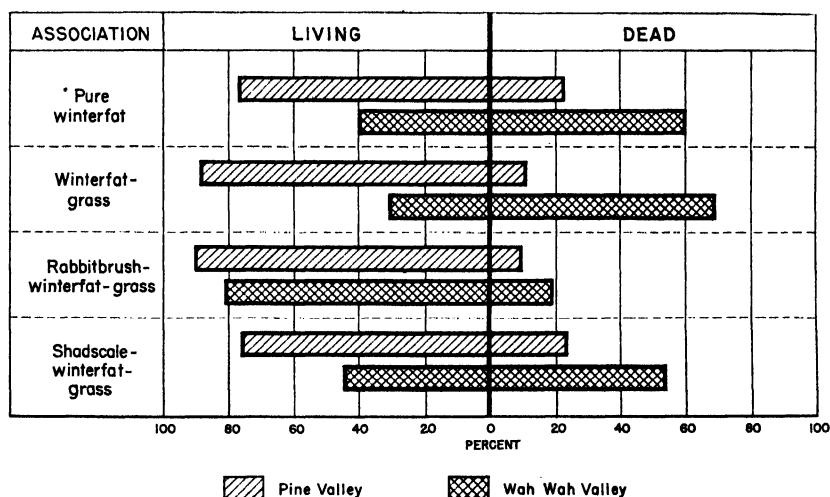


FIGURE 11. Percentages of living and dead winterfat plants in the plant associations indicated in Pine and Wah Wah Valleys.

WINTERFAT-RABBITBRUSH

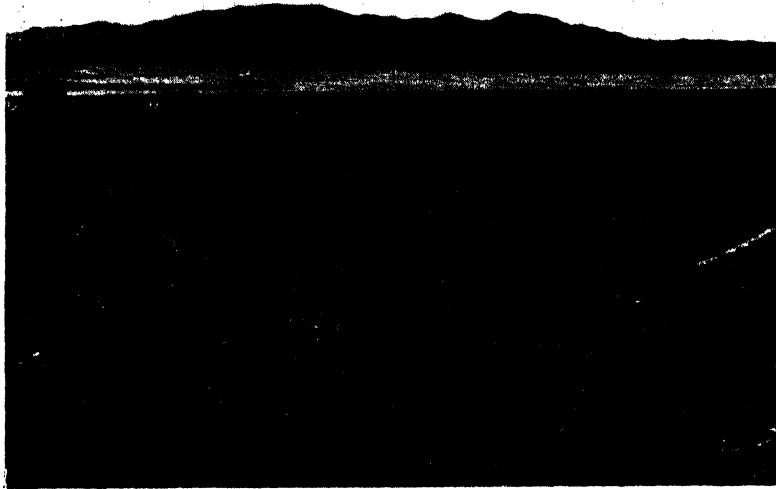
Winterfat and small rabbitbrush are considered together because records from old residents supported by an abundance of ecological data show that the desert valleys during the early history of grazing were dominated by the winterfat-grass association; the hundreds of square miles of small rabbitbrush today is a product of the present century; and this species is establishing itself rapidly on sites from which winterfat is disappearing at an alarming rate (fig. 12).

Winterfat reaches its best expression in almost pure stands on broad, flat watercourses of the valleys, where the soil is somewhat better watered by accumulations from the melting snows of spring or torrential downpours of summer rain. In addition, as one component of a mixed stand, it extends normally in all directions up the gentle valley slopes to the mixed shadscale associations.

Rabbitbrush invasion has been most active on the more sandy soils and least active on the naturally irrigated lands of the watercourses. Competition between the two species for possession of the desert

has been severe (8), as is shown by the present sharp lines which separate them and the almost total dominance of each in its own territory. Small gray circular islands of winterfat in green stretches of rabbitbrush or a wedge of the latter indenting the margin of an expanse of winterfat are characteristic features of these plant types. This situation is perhaps explained by slight physical and chemical soil differences, although hardpans and a high saline content of the subsoil characterize both conditions.

The rapid increase of young small rabbitbrush plants and the high mortality of palatable forage species in Wah Wah Valley establish grazing as a major cause of deterioration in the vegetation. In view of the great difference in the palatability of the two species, together



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FIGURE 12.—Rabbitbrush (dark-colored shrubs) invading weakened winterfat areas in Antelope Valley not far from Pine Valley transect.

with the difference in intensity of grazing use between Pine and Wah Wah Valleys, the following facts gained from the study are significant:

(1) The mortality of small rabbitbrush in both valleys was less than 10 percent, but that of winterfat was 23 percent in pure winterfat communities in Pine Valley, 59 percent in Wah Wah Valley, and even higher in the winterfat-grass association (fig. 11).

(2) In Pine Valley 30 percent of the rabbitbrush, and in Wah Wah Valley 43 percent, were produced during the decade 1927-36. The same period accounted for 1 percent of the winterfat in Pine Valley, and none on the sites studied in Wah Wah Valley (fig. 13).

(3) In pure stands of winterfat in Pine and Wah Wah Valleys only 1 percent of the plants had less than 10 vascular rings, whereas in pure stands in Snake Valley about 25 miles to the north, where grazing has been much lighter than in either of the other valleys, 8 percent were in this class (fig. 14).

(4) On the 200 square miles or so occupied by these plants growing together in association in Pine and Wah Wah Valleys there is an average of one winterfat plant to the square foot. Of these winterfat

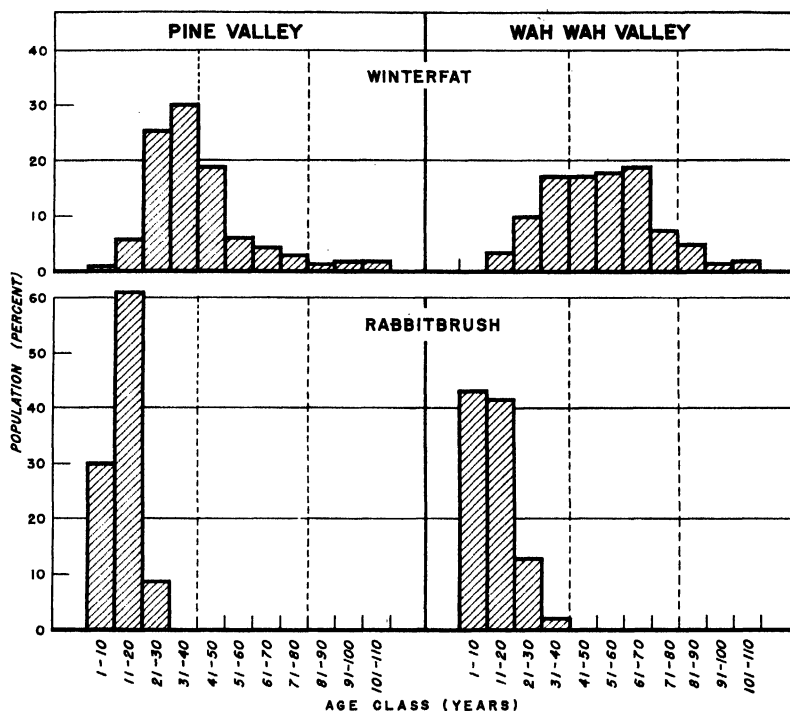


FIGURE 13.—Winterfat and rabbitbrush plants in mixed association, classified by vascular rings, Pine and Wah Wah Valleys.

plants, 10 percent in Pine Valley and 19 percent in Wah Wah Valley are dead (fig. 11). The live plants furnish a ground cover of 1.5 percent of a total ground cover of 1.7 percent in Pine Valley and of 0.09 percent of 0.36 percent in Wah Wah Valley.

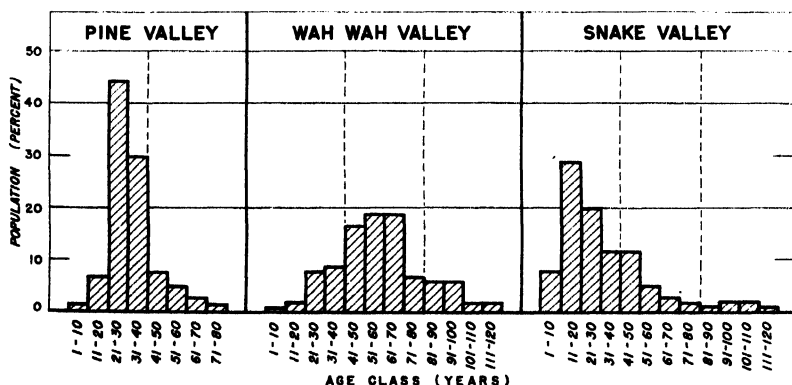


FIGURE 14.—Winterfat plants, classified by the number of vascular rings, form nearly pure populations in Pine, Wah Wah, and Snake Valleys. Young plants are most abundant in Snake Valley where the scarcity of watering places limits grazing to the periods when snow is available for stock water.

(5) The lowest mortality for winterfat in both valleys is found in the rabbitbrush communities where the winterfat plants are few. The low palatability of the dominant shrubs makes forage cover as a whole relatively unpalatable to livestock. It is therefore not heavily grazed.

Small rabbitbrush plants in these plant types are predominantly 20 years old or less (fig.13), indicating a recent invasion. The shrub is normally long-lived, however, as evidenced by the fact that on one area 26 percent of the plants are more than 30 years of age and 4 percent more than 40 (fig.15).

A puzzling feature of the rabbitbrush invasion of winterfat associations is its apparent arrest. In spite of the 100 square miles of land open to the invasion because of the depletion of the natural cover, few invading young rabbitbrush plants are found beyond sharply delimited outposts. The explanation of this paradox prob-

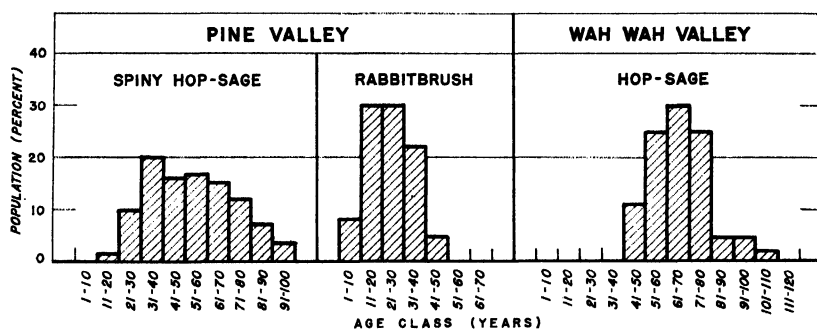


FIGURE 15.—Spiny hop-sage and small rabbitbrush on favorable sites in Pine and Wah Wah Valleys, classified by the number of vascular rings.

ably lies in the present widespread and dense occupation by Russian-thistle, which within the last 15 years has overrun all badly deteriorated areas.

Russian-thistle is both a blessing and a curse to the desert; its seeds germinate at any period of the growing season when moisture is available, and the plants in a short time afford protection against wind erosion; but on the other hand, seedlings of more palatable perennial species under heavy grazing appear practically unable to compete with it. It is a hardy invader in good years, but like all annuals, native or exotic, cannot maintain a plant cover in years of severe drought. In Wah Wah Valley it varied from 20 percent of the ground cover in 1932 to practically nothing in 1934.

HOP-SAGE

One of the most palatable desert shrubs is spiny hop-sage. The root chart (fig. 6) and table 2 show this species to be definitely restricted in habitat; its total area on the desert was never large. The soils in which it thrives are the most free from salt of those supporting any major association. Its preferred habitat seems to be in sands free of hardpans, often granitic, and occasionally watered by intermittent streams.

Approximately 85 percent of the plants in what was classed as pure hop-sage stands in Wah Wah Valley are dead (fig. 16), and the remain-

der nearly so. The number of vigorous plants throughout most of the open winter range has been greatly reduced by overgrazing. Under lighter forage use in Pine Valley, 37 percent of the plants in pure stand are dead and the others severely injured. Since no reproduction whatever was found to have occurred during the last 10 years

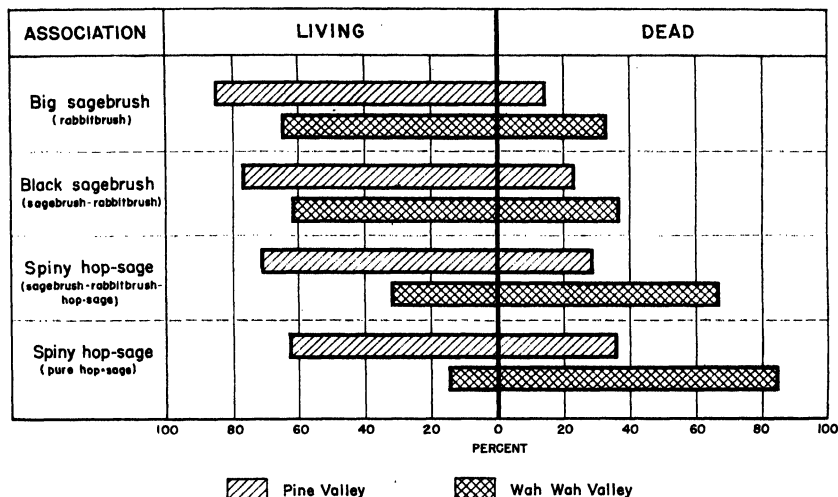


FIGURE 16.—Percentages of living and dead plants of big sagebrush, black sagebrush, and spiny hop-sage in various plant associations in Pine and Wah Wah Valleys.

(fig. 15) and but 1 percent during the last 20 years, the conclusion is that the species cannot withstand even moderately heavy use and that the early extermination of this plant is almost certain. Numerous dead and dying plants of blue grama and galleta bear witness that the hop-sage association, once rich in both quality and quantity of forage, is now on the verge of total extinction (fig. 16).



FIGURE 17.—A hop-sage association in Pine Valley, showing the hedged shrubs and stubble of grama grass.

Small rabbitbrush is invading the hop-sage association and will in all probability, under present grazing practices soon succeed that species on many tracts. On an area used for continuous bedding of sheep in a hop-sage association in Pine Valley, rabbitbrush has supplanted all of the original palatable vegetation.

SAGEBRUSH

Two sagebrushes, notably different in their choice of habitat and in forage value, occupy these desert lands. Big sagebrush, relatively unpalatable and little grazed except under conditions of cold and deep snow or where other forages are lacking, is confined largely to

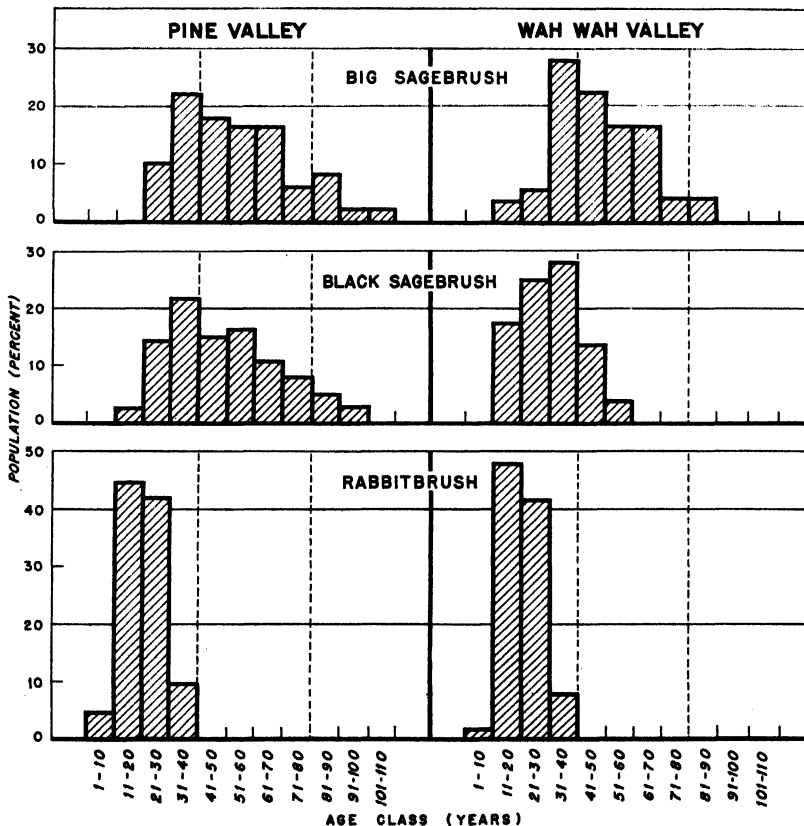


FIGURE 18.—Big sagebrush, black sagebrush, and rabbitbrush growing in association in Pine and Wah Wah Valleys, classified according to the number of vascular rings.

the deep porous soil of the valley uplands, where it makes up the greatest density of plant cover of any association on the desert. The highly palatable black sagebrush constitutes the chief understory of the juniper-piñon association of the hillsides and is also abundant along sides of shallow drainage channels at the upper valley edges. Both sagebrushes grow best on soils free from salt, at least to the ordinary feeding depth of the roots. Usually, black sagebrush occurs on

shallow, rocky soil in contrast with the arable, porous soil preferred by big sagebrush.

Taxonomically, these two sagebrushes are difficult to distinguish. The most constant difference is the light-colored flower stalk and the numerous heads of the large species, as contrasted with the brownish flower stalk and few heads of the smaller. Sheep, however have little difficulty in distinguishing them; black sagebrush is almost invariably closely browsed, while big sagebrush, even when immediately adjacent, often remains almost untouched.

A comparison of the mortality records for the two species in both Pine and Wah Wah Valleys is given in figure 16. Two significant facts are apparent: (1) Mortality is lower for both in the less heavily



FIGURE 19.—Big sagebrush reduced in size by grazing and invading rabbitbrush on a bed ground in Wah Wah Valley.

grazed Pine Valley than in Wah Wah Valley; and (2) the mortality of the more palatable black sagebrush is somewhat greater in both valleys.

Stockmen who have operated sheep outfits on the desert for 20 years or more declare that black sagebrush, the most palatable of all desert shrubs with the possible exception of spiny hop-sage, has decreased tremendously. The principal remnant of any consequence in these two valleys is in the juniper belt, where the plant growth is ordinarily covered with snow in midwinter and is thus protected for a season from excessive grazing.

Small rabbitbrush is, in volume, an important component of these plant types. The age-class data (fig. 18) reveal it as an invading species, just as in other associations important to desert grazing, and indicate that here, too, this invader of low forage value is taking possession of immense areas that formerly supported palatable shrubs (fig. 19).

Big sagebrush communities are chosen more frequently than black sagebrush as bedding grounds for sheep inasmuch as big sagebrush occupies the uplands which are preferred for this purpose. They are

in this way exposed to longer periods of grazing, and so include even fewer plants under 20 years of age (fig. 18). The species is reproducing in some cases, however, owing to the favorable environmental factors. It has replaced the more palatable winterfat in one large part of Wah Wah Valley. A rather extensive young stand was found also in Pine Valley.

HORSEBRUSH AND GREASEWOOD

Horsebrush occurs in small areas in both valleys, as does greasewood. Horsebrush species are highly unpalatable and harmful to sheep, but because the plants of this genus are eaten under pressure of great hunger they deserve critical study. Most of them occur on shallow upland soils otherwise similar to the shadscale-grass habitats. Many dead Indian ricegrass clumps in the association suggest that horsebrush, like small rabbitbrush, is an invading species. Greasewood, because it occurs only on saline soils with high water tables, is of little consequence in Pine and Wah Wah Valleys.

OPEN FORESTS

The Pine Valley transect passes from the desert-shrub through a juniper-piñon belt, at the upper limits of which it traverses dense stands of singleleaf piñon. At higher elevations it passes through a white fir-aspen association.

Piñon and juniper are overstory members of a single association. The piñon dominates the higher elevations, but at the lower limits of



FIGURE 20.—Juniper in Wah Wah Valley, the lower branches of which have been nibbled by sheep. Note the remains of sagebrush and the invasion of rabbitbrush.

the association the juniper occurs in nearly pure stands which ultimately give way to the valley shrub types (fig. 20). Midway, both occur as commensals in a mixed overstory. Sagebrush species are the principal understory shrubs, with big sagebrush occupying the alluvial

deeper soils and black sagebrush dominating shallow, rocky ones. Other shrubs encountered frequently in this association include serviceberry, dwarf goldenbush, curleaf mountain-mahogany, green ophedra, broom snakeweed, globe cactus, and bitterbrush.

Grasses, probably abundant in the juniper-piñon association before the days of excessive grazing, still present a great assortment of species. Of these, the most important are bluebunch wheatgrass, red three-awn, blue grama, blue wild-rye, galleta, Indian ricegrass, squirreltail, and needle-and-thread grasses.

In contrast with the purely desert types, the juniper-piñon association exhibits a striking seasonal periodicity of its flora. In favorable years, a large assortment of early spring annuals are supported on the foothills, followed in turn by summer and fall herbaceous forms. Principal among this weed flora are rockcresses, sandworts, aster, astragalus, wild-cabbage, Douglas morningbrides, hedgehog cactus, wild-daisy, eriogonum, slender buckwheatbrush, gilijs (including tin piute), globe cactus, pricklypear, pentstemon, phlox, groundsels, rock goldenrod, and globemallows.

Ponderosa pine is not common to desert mountains, but local stands exist in a few places. The species is found normally at elevations above the piñon-juniper belt and, as isolated communities, occurs at about 9,000 feet elevation all along the Wah Wah range. Associated with it are Douglas fir, white fir, and aspen. The summit of Indian Peak where the Pine Valley transect terminates is for the most part covered with stony talus and consequently the slopes are generally barren. No ponderosa pine occurs there, but white fir, Douglas fir, and aspen grow in sheltered places.

SUMMARY AND CONCLUSIONS

The vegetation in Wah Wah Valley, Utah, which has been grazed for about 70 years, and severely so for about 50 years, is compared with that in the nearby Pine Valley where, owing to the scarcity of stock water, grazing use was heavy only when snow was available until about 20 years ago, when watering places were artificially developed. The plant cover of these two neighboring valleys, comparable in size, in physiographic features, in soils, and in forage-plant associations, differed mainly as a result of the duration and intensity of livestock grazing to which they had been subjected. In order to compare the effects on vegetation of these two types of grazing use, the density, kind, and condition of the forage plants in the two valleys as well as the prevalent soil conditions, were studied on a long transect across each valley.

Palatable perennial grasses, formerly conspicuous in all the desert-shrub associations, today are few in number and low in vigor where the grazing has been severe during the protracted period of approximately 50 years. In comparison with those of low palatability, species of high palatability have suffered (1) a greater loss in density of plant cover, (2) a higher plant mortality, (3) a greater decrease in reproduction, and (4) a sharper decline in general vigor.

Palatable shrubs such as winterfat, shadscale, spiny hop-sage, and black sagebrush have also suffered in the same respects as have the grasses under prolonged heavy grazing. Of the forage shrubs, on which ring counts were made, very few plants were less than about 20 years of age. Grasses also showed low reproduction during a long period of years, although the length of the period was not susceptible to accurate determination.

The root systems of the desert forage species studied were found to be largely limited to the upper 30 cm. of soil where the salt content rarely exceeds 1,000 parts per million. The root systems of big sagebrush and spiny hop-sage extend much deeper than this. Associations in which these species are dominant are limited to areas where even the subsoils have a low salt content.

The naturally sparse plant cover when thinned and weakened by unrestricted grazing has permitted heavy wind erosion, and on a few of the worst areas, the beginning of dune formation.

Small rabbitbrush, low in palatability to livestock and once rare on the desert, has on heavily grazed areas replaced to an alarming extent the more palatable species, but is still rare where the palatable species are vigorous.

Russian-thistle, introduced to the desert two decades ago, is not a serious competitor in vigorous shrub communities, but on heavily grazed areas depleted of vegetation it has taken possession and apparently retards reproduction of desert shrubs.

Impoverishment of desert forage, attributed by many to the drought of 1928-35, was not so markedly apparent after the drought of 1897-1904, which was of as long duration and, measured in total precipitation, more severe. The evidence gained from the study of these plant associations does not support the theory that drought is the sole or even the chief cause of present deterioration and depletion of the range. Instead, it points unmistakably to unrestricted grazing as the chief cause of loss of grazing values, invasion of inferior species, and the gradual crowding out of the most palatable range plants.

The data show clearly that the heavy utilization of the forage by livestock must be relaxed in order to provide for restoration of the range to normal producing power and for its subsequent maintenance. The original presence of accessible stock water in Wah Wah Valley permitted heavy range use in the fall and in the spring as well as during winter. The artificial development about 20 years ago of stock water in Pine Valley, formerly grazed only when snow was available, has permitted heavy use in fall and spring as well as in winter since that time. The result during the last few years has been to set in operation on the forage plants the deteriorating forces of overuse.

Permanent and vigorous forage production on the winter ranges will require the sort of range management that avoids complete utilization of the current year's growth and that will give some relaxation in the degree of use during fall and spring. Development of wells and other stock-watering places needs to be accompanied by control and management of livestock lest the new watering places become centers of yet further forage deterioration.

COMMON AND SCIENTIFIC NAMES OF PLANTS DISCUSSED

Aspen.....	<i>Populus tremuloides aurea</i>
Aster.....	<i>Aster engelmanni</i>
Big sagebrush.....	<i>Artemisia tridentata</i>
Bitterbrush.....	<i>Purshia tridentata</i>
Black sagebrush (black sage).....	<i>Artemisia nova</i>
Bluebunch wheatgrass.....	<i>Agropyron spicatum</i>
Blue grama.....	<i>Bouteloua gracilis</i>
Blue wild-rye.....	<i>Elymus glaucus</i>
Broom snakeweed.....	<i>Gutierrezia sarothrae</i>
Curleaf mountain-mahogany.....	<i>Cercocarpus ledifolius</i>
Douglas fir.....	<i>Pseudotsuga taxifolia</i>
Douglas morningbrides.....	<i>Chaenactis douglasii</i>
Dwarf goldenbush.....	<i>Aplopappus nanus</i>
Eriogonum.....	<i>Eriogonum heracleoides</i> and <i>E. racemosum</i>
Galleta.....	<i>Hilaria jamesii</i>
Gilia.....	<i>Gilia</i> spp.
Globe cactus.....	<i>Pediocactus simpsonii</i>
Globemallow.....	<i>Sphaeralcea caespitosa</i> , <i>S. coccinea</i> , and <i>S. grossulariaefolia</i>
Goldenweed.....	<i>Aplopappus falcatus</i>
Gray molly.....	<i>Kochia vestita</i>
Greasewood.....	<i>Sarcobatus vermiculatus</i>
Green ephedra.....	<i>Ephedra viridis</i>
Groundsels.....	<i>Senecio</i> spp.
Hedgehog cactus.....	<i>Echinocereus mojavensis</i>
Horsebrush.....	<i>Tetradymia</i> spp.
Indian ricegrass.....	<i>Oryzopsis hymenoides</i>
Jointfir. (See Green ephedra.)	
Needle-and-thread.....	<i>Stipa comata</i>
Oreocarya.....	<i>Oreocarya confertifolia</i> and <i>O. depressa</i>
Pentstemon.....	<i>Pentstemon palmeri</i> and other species
Phlox.....	<i>Phlox longifolia</i> and other species
Ponderosa pine.....	<i>Pinus ponderosa</i>
Pricklypear.....	<i>Opuntia</i> spp.
Red three-awn.....	<i>Aristida longiseta</i>
Reverchon three-awn.....	<i>Aristida glauca</i>
Rockcress.....	<i>Arabis</i> spp.
Rock goldenrod.....	<i>Solidago petradoria</i>
Russian-thistle.....	<i>Salsola pestifer</i>
Sand dropseed.....	<i>Sporobolus cryptandrus</i>
Sandwort.....	<i>Arenaria</i> spp.
Serviceberry.....	<i>Amelanchier alnifolia</i>
Shadscale.....	<i>Atriplex confertifolia</i>
Singleleaf pifion (pifion pine).....	<i>Pinus monophylla</i>
Slender buckwheatbrush.....	<i>Eriogonum microthecum</i>
Small rabbitbrush.....	<i>Chrysothamnus stenophyllus</i>
Small sagebrush. (See Black sagebrush.)	
Spiny hop-sage.....	<i>Grayia spinosa</i>
Squirreltail.....	<i>Sitanion hystrix</i>
Tin piute.....	<i>Gilia aggregata</i>
Utah loco.....	<i>Astragalus utahensis</i> and other species
White fir.....	<i>Abies concolor</i>
Wild-cabbage.....	<i>Caulanthus crassicaulis</i>
Wild-daisy.....	<i>Erigeron caespitosus</i>
Winterfat (white sage).....	<i>Eurotia lanata</i>

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TOXICITY TO SHEEP OF LEAD ARSENATE AND LEAD ARSENATE SPRAY RESIDUES¹

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INTRODUCTION

Occasionally reports are received at the Washington station of the illness or death of livestock, principally cattle and sheep, that have been pastured in sprayed orchards during the fall. Many of these losses have occurred where veterinary service was not available and have been attributed to anthrax or to some other acute infectious disease. These diagnoses, however, have not been confirmed by members of the veterinary profession or by laboratory findings.

During the last 2 years the authors have investigated two such outbreaks, one of which resulted in the loss of approximately 1,000 sheep and the other in the loss of 23 feeder steers (8a).³ The post-mortem pathology and the essentially negative nature of the bacteriological findings, together with the case histories, pointed to acute chemical poisoning rather than to an acute infectious disease.

The orchard grasses from the area on which the sheep were known to have fed showed on analysis 0.44 percent of arsenic (As) and 1.44 percent of lead, which is more than 4,000 times the established tolerance for foods. The leaves of the trees on which the steers are known to have fed contained 0.0045 percent of arsenic.

Analyses of the rumen contents of the last of the poisoned animals to die and of moribund survivors revealed the presence of sufficient lead and arsenic to account for 30 gm. of lead arsenate in the sheep and 38 gm. in the steers. Since sufficient lead arsenate had been absorbed to cause severe illness or death and some had been eliminated from the body, it is evident that considerably larger amounts had been ingested.

Approximately 15 percent of the poisoned sheep and 80 percent of the poisoned steers made at least a temporary recovery from the effects of the lead arsenate. If it be assumed that some of the animals which were made ill, but which did not succumb within the following 3 months, consumed almost as much as did the last of the animals to die of acute poisoning, the amounts of lead arsenate consumed by these survivors were considerably greater than the reported minimum lethal doses of lead arsenate for sheep and cattle.

REVIEW OF THE LITERATURE

The literature contains numerous references to poisoning with lead and with arsenic. For bibliographies the reader is referred to Osborn

¹ Received for publication August 7, 1939. Published as Scientific Paper No. 414, College of Agriculture and Experiment Station, State College of Washington.

² The writers express their appreciation to D. Finch and L. Murphy for assistance in caring for the experimental animals, and to F. Van Amburgh for the analytical work.

³ Italic numbers in parentheses refer to Literature Cited, p. 328.

and Pease,⁴ Pease and Osborn,⁵ and Aub (2). Most of the published work, however, is based upon the toxicity of lead and arsenic fumes or dust; the toxicity of lead and arsenic compounds other than lead arsenate; the effects of these compounds upon rats and other laboratory animals and upon dosages far below the levels known to have been consumed by the sheep and cattle that died after having grazed upon the forage in heavily sprayed orchards. In most of the experiments in which large animals were used, the experimental animals were allowed to graze areas of lead arsenate-sprayed forage. From such experiments, the exact amounts of lead arsenate eaten by the animals cannot be ascertained.

Data obtained from inhalation of lead and arsenic dust or fumes probably are not applicable to the oral administration of these substances.

Perhaps the lower incidence of plumbism in animals consuming lead-contaminated forage than in people exposed to lead in the industries may be explained by the difference in the ways in which exposure occurs. Lanza (8) pointed out (8, p. 85) that—

most of the industrial exposure arises from dust and fumes that are breathed into the lungs and upper respiratory tract, where absorption and excretion involve entry into the systemic circulation. Lead that is ingested may be excreted unchanged and, even if absorbed, may be carried to the liver and excreted in the bile.

Cardiff (3) believes that the poisoning of horses, when hay from heavily sprayed orchards is fed, results from the inhalation of the dried spray residues rather than from the consumption of the lead arsenate.

Thomas and Shealy (15) found that while lead arsenate will reproduce death in chickens when fed in large quantities, a chicken may consume as much as 13 grains per day for 60 days without suffering any ill effects.

Coulson, Remington, and Lynch (4) found that rats fed shrimp which naturally contained 17.70 parts per million of arsenic stored only 0.13 mg. of arsenic in 3 months, while in a parallel experiment rats which received a similar amount of As_2O_3 in their diet stored 3.73 mg. After as long as 12 months on these diets, neither group showed any evidence of poisoning.

Although Talbert and Tayloe (14) do not feel that their data are sufficient to permit conclusions regarding lead poisoning from the consumption of sprayed apples, they do state that there is little likelihood of a person consuming as spray residue on apples enough arsenic at one time or over an extended period to be injurious.

Husband and Duguid (7) found that cattle survived 20 grains of arsenic fed as sodium arsenite. Thirty grains killed in some cases and larger amounts were fatal. Cattle died after consuming pasture grass sprayed with 1.5 pounds of sodium arsenite per acre. Arsenic was found in the intestinal contents and in several organs. Husband and Duguid state that analyses of the omasum and kidney give more reliable information than those of other organs in arsenic poisoning.

One of the earliest experiments in which exact amounts of lead arsenate were fed was conducted by Paige (10), who used a lead arsen-

⁴ OSBORN, R. A., and PEASE, V. A. SELECTED REFERENCES ON THE TOXICITY OF ARSENIC, 1901-1934. U. S. Bur. Chem. and Soils, Food Res. Div. 45 pp. 1934. [Mimeographed.]

⁵ PEASE, V. A., and OSBORN, R. A. SELECTED BIBLIOGRAPHY ON METALLIC CONTAMINATION OF FOODS, WITH SPECIAL REFERENCE TO CONTAINERS AND COOKING UTENSILS. U. S. Bur. Chem. and Soils, Food Res. Div. 87 pp. 1936. [Mimeographed.]

ate paste somewhat different in composition from that now applied to orchards. Since the lead arsenate paste contained about 9 percent of arsenic and 32.5 percent of lead, the quantities of the paste that he administered should be reduced to approximately one-half his stated value to be comparable to modern dry lead arsenate.

All the cattle that Paige used were tuberculous, which may have decreased their ability to withstand toxic doses and to recover after they were poisoned. In his experiments Paige fed a 600-pound cow 1 gm. of lead arsenate paste per day for 26 days and observed no symptoms of lead poisoning. Following a 4-day interval she was again given 1 gm. per day and died after 10 days. A 1,015-pound cow given 184 gm. of lead arsenate paste in daily doses of from 1 ½ to 3 gm. over a period of 117 days, made an apparent recovery, but a single dose of 50.4 gm. 43 days later resulted in violent purgation and inappetence. She was autopsied 19 days later. Another cow weighing 600 pounds survived a single dose of 28.35 gm. of lead arsenate paste, and 96 days later was started on another series of tests in which 105 gm. were fed in 57 days. Two days later she was given a single dose of 19.8 gm. and slaughtered 9 days thereafter. Paige reports (10, p. 190):

Principal lesions: small tubercular nodule in lobe of lung; indications of the irritant and poisoning action of arsenate of lead few. Blackening of the gums about the incisor teeth quite pronounced in this animal; not found in any of the others.

Green and Dijkman (6) found that in most cases the oral administration of 0.2 to 0.3 gm. of arsenic trioxide, supplied as sodium arsenite, produced no symptoms of poisoning in sheep and that death was uncertain when less than 0.5 gm. was given.

Seddon and Ramsay (12) studied the toxicity of various compounds of lead and of arsenic for sheep by mixing the drug with small quantities of bran and sirup and administering it as an electuary. For 80- to 100-pound sheep they found fatal doses to be as shown in table 1. Of especial interest are the differences in the amounts of arsenic contained in the toxic and the nontoxic doses when the arsenic was given in various chemical combinations. The data indicate the more soluble compounds of arsenic to be the more toxic.

TABLE 1. — *Lethal doses of various arsenic and lead compounds when administered to sheep in bran and sirup as an electuary*

[From work of Seddon and Ramsay (12)]

Dose and compound administered	Size of dose		Poison contained in dose		
			As ₂ O ₃	As	Pb
	Grains	Grams	Grams	Grams	Grams
Fatal doses:					
Sodium arsenite.....	13.3	0.862	0.577	0.437
Paris green.....	20.0	1.296	.715	.542
Arsenic acid.....	13.3	.862	.551	.417
Lead arsenate.....	60.0	3.888	1.251	.947	2.317
Lead oxide.....	180.0	11.664	9.691
Lead acetate.....	270.0	17.496	9.321
Nontoxic doses:					
Sodium arsenite.....	6.6	.428	.287	.217
Arsenic acid.....	6.6	.428	.274	.208
Lead arsenate.....	40.0	2.592	.835	.632	1.545
Lead acetate.....	216.0	13.997	7.457
Lead sulfate.....	60.0	3.888	2.655

O'Kane, Hadley, and Osgood (9) fed lead arsenate to sheep and other animals. The lead arsenate was sprayed on green foliage which the sheep consumed directly. The exact amount consumed was, therefore, not known. With two sheep that were estimated to have received a little over 3 gm. of lead arsenate each per day for 45 days, any symptoms of poisoning disappeared by the end of 30 days. Sheep receiving 7 gm. per day showed definite symptoms of poisoning, and by the fifth day the feeding was discontinued. These animals recovered in about 3 days. Another lot received about 12 gm. (estimated) per day of lead arsenate. These animals showed prompt and definite symptoms of poisoning. One died on the fourth day. The lead arsenate feeding was discontinued with the other sheep, which appeared quite normal after 4 days and finally recovered.

Reeves (11) reports that sheep were poisoned by 4 to 8 grains of arsenic when it was fed daily.

EXPERIMENTAL PROCEDURE

A series of experiments was outlined in which sheep were fed lead arsenate at various levels daily. The sheep used were healthy, vigorous lambs weighing approximately 80 pounds (36.29 kg.). Each animal was maintained in an individual metabolism crate at the College of Veterinary Medicine throughout the experiment. The basal ration fed to all experimental animals consisted of rolled oats 3 parts, rolled barley 4 parts, and alfalfa hay 14 parts, with salt ad libitum. Since fallen apples are usually consumed by animals pastured in orchards, a measured quantity of Jonathan apples was included in the ration of some of these animals. The animals were accustomed to the metabolism crates and to the basal ration and were making daily gains before the administration of arsenic was started.

During the experimental period the urine and feces were collected daily, sampled, placed in 2-quart jars to which thymol was added, and the samples placed in cold storage. The daily feed and water consumption were accurately measured. The daily dose of lead arsenate for each animal was divided into two portions which were placed in gelatin capsules and given morning and night, so that the exact amount of lead arsenate taken into the body was definitely known. The lead arsenate used was a well-known brand which is widely used in orchard spraying in Washington State. It contains 59.6 percent of lead and 21.5 percent of arsenic.

For the determination of lead the tentative methods of the Association of Official Agricultural Chemists (1) were followed except that in preparing the samples for analysis nitric and perchloric acids were used according to methods developed in the laboratory of the Division of Chemistry by Gerritz (5). The lead was isolated by direct diphenylthiocarbazone extraction or, when necessary, the lead was precipitated and isolated as the sulfide, dissolved in 1 percent nitric acid and extracted with diphenylthiocarbazone. The quantitative readings were then made by colorimetric comparison according to the method developed for spray-residue determination. Arsenic was determined on an aliquot of the nitric and perchloric acid-digested sample either by the bromate or the Gutzeit method, depending upon the quantity of arsenic present.

EXPERIMENT 1

In the first experiment three sheep were fed 2 g. of lead arsenate daily. Two of these received 2 pounds of apples each day in addition to the basal ration and the third received only the basal ration. One of the sheep that received apples and the sheep that received only the basal ration died on the fourth day after each had consumed a total of 7 g. of lead arsenate. The remaining sheep, which had received apples, died after 1 week, having consumed a total of 15 g. of lead arsenate.

On autopsy the sheep that died on the fourth day were found to have hemorrhagic erosions of the rumen wall, which extended into the reticulum. Severe enteritis was present, especially in the duodenum. The lungs were pneumonic. In the third sheep the lesions were similar to those in the other two except that the kidneys had a metallic sheen.

As soon as the lead arsenate was given to the sheep, their feed consumption decreased by approximately 80 percent, and the amount of water consumed was also markedly reduced.

The daily excretion of lead and arsenic, expressed in milligrams, is shown in table 2. While there are variations in the quantity of lead and arsenic eliminated from day to day and in the quantity eliminated by different sheep, these data do not show any correlation between the inclusion of apples in the diet and the rate of lead or arsenic elimination. The data are made more difficult to interpret by the marked reduction in feed and water intake and a somewhat parallel reduction in the amount of feces and urine following the administration of the lead arsenate.

Much of the arsenic eliminated was through the urine, although the feces contained appreciable amounts. From 44 to 48 percent remained in the digestive tract at the time of death, while from 54 to 60 percent of the total amount of arsenic fed to these sheep was accounted for by analysis of the contents of the digestive tract, the liver, kidneys, and the wool.

More lead was eliminated from the body through the feces than through the urine. From 60 to 77 percent remained in the digestive tract, while from 62 to 79 percent of the lead consumed was accounted for by analysis of the digestive tract, the liver, and the kidneys.

TABLE 2.—Total lead arsenate ingested by sheep fed 2 gm. daily; quantity of arsenic and lead excreted daily; and total quantity found at autopsy

Sheep	Total lead arsenate ingested ¹		Arsenic and lead excreted—												Total found at autopsy in—							
			Mar. 16		Mar. 17		Mar. 18		Mar. 19		Mar. 20		Mar. 21		Mar. 22		Liver		Kidney		Stomach	
	As	Pb	As	Pb	As	Pb	As	Pb	As	Pb	As	Pb	As	Pb	As	Pb	As	Pb	As	Pb	As	Pb
	Grams	Grams	Mili-grams	Mili-grams	Mili-grams	Mili-grams	Mili-grams	Mili-grams	Mili-grams	Mili-grams	Mili-grams	Mili-grams	Mili-grams	Mili-grams	Mili-grams	Mili-grams	Mili-grams	Mili-grams	Mili-grams	Mili-grams	Mili-grams	Mili-grams
Urine:																						
2 ¹	3.24	8.95	34.2	2.4	42.0	1.6	42.6	1.4	58.8	3.0	18.3	1.1	45.7	3.0	38.8	16.0	7.3	2.9	1.7	1.2	1,599.0	8,988.0
3.....	1.51	4.18	35.1	2.9	18.1	1.2	—	—	—	—	—	—	—	—	—	—	27.3	7	1.7	1.4	716.0	3,280.0
4 ¹	1.51	4.18	25.8	4.1	31.7	4.5	—	—	—	—	—	—	—	—	—	—	7.8	6	1.3	1.1	696.0	2,512.0
Feces:																						
2 ¹	—	—	9.2	54.9	6.0	26.6	27.3	114	25.2	80.6	—	—	—	—	—	—	—	—	—	—	—	—
3.....	—	—	2.0	36.5	7.2	32.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4 ¹	—	—	1.2	30.9	3.4	54.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

¹ Lead arsenate first fed Mar. 15, 1933.² Apples included in ration.

EXPERIMENT 2

Since the daily administration of 2 gm. of lead arsenate had proved lethal to three sheep in from 2 to 7 days, a second experiment was conducted in which the amounts of lead arsenate given daily were 1, 0.5, and 0.25 gm. Two sheep, one of which received the basal ration only and the other received apples in addition, were placed on each level. A seventh sheep was included as a control.

One of the sheep that received 1 gm. of lead arsenate daily and apples as part of the ration died in 6 days; the other sheep that received 1 gm. of lead arsenate daily and only the basal ration died in 7 days. The lethal amount of lead arsenate fed at the rate of 1 gm. per day was, therefore, 6 or 7 gm. These figures represent 1.29 and 1.51 gm. of arsenic (As) (equivalent to 1.70 and 1.99 gm. if expressed as As_2O_3). It should be noted that this is approximately the same total quantity that killed two of the three sheep in experiment 1 where 2 gm. of lead arsenate were fed daily. The 1.5 gm. of arsenic (As) for an 80-pound sheep is equivalent to 41 mgm. per kilogram, or 192 mgm. of lead arsenate per kilogram.

One of the sheep receiving 0.5 gm. of lead arsenate per day died after 35 days, having consumed a total of 17.5 gm. of lead arsenate, equivalent to 3.7 gm. of arsenic (As). The other sheep receiving 0.5 gm. of lead arsenate per day died after 79 days, having consumed 39.5 gm. of lead arsenate, equivalent to 8.4 gm. of arsenic.

One of the sheep receiving 0.25 gm. of lead arsenate daily died after 35 days, having received 8.75 gm. of lead arsenate containing 1.9 gm. of arsenic. The other sheep survived 94 days on this level, when the lead arsenate feeding was discontinued, and 10 months later when slaughtered was in good condition and showed no evidence of injury. This sheep consumed 23.5 gm. of lead arsenate during the 94 days representing 5 gm. of arsenic. The excretion of lead and arsenic by the sheep in experiment 2 is shown in table 3.

As in experiment 1, the amount of arsenic eliminated daily in the urine varied widely, and there appeared to be no correlation between the amount fed per day and the amount eliminated. Essentially the same is true with regard to the elimination of arsenic and lead in the feces, although in general more arsenic was eliminated per day in the feces than in the urine. In the feces the ratio of the amount of lead eliminated to the amount of arsenic eliminated was much above the ratio of these two elements in lead arsenate. The actual weight of arsenic eliminated per day in the feces was greater than in the urine in experiment 2; the reverse was true in experiment 1.

Table 4 summarizes the total amount of lead and arsenic eliminated and the total remaining in the stomach and other organs.

The data show that only a small proportion of the ingested lead arsenate was eliminated. Of the four sheep in experiment 2 that lived for 35 days or more, one eliminated 16 percent of the arsenic intake, while two eliminated 4 and 5 percent. The elimination of lead varied from 46 to 12 percent.

Thirty-three to forty-two percent of the arsenic accounted for was found in the stomach contents, while 17 to 24 percent of the lead was found there. The total amount of arsenic accounted for ranged from 10 to 28 percent of the amount ingested while the total amount of lead that could be traced ranged from 32 to 61 percent. Analyses were made of the liver, kidneys, heart (table 4), and gall bladder, and in one case of the lungs, ribs, and other bones, bone marrow, and wool (table 5). The concentration in all the parts examined was small as compared with that in the stomach contents, which accounted for 4 to 38 percent of the arsenic ingested and 13 to 49 percent of the lead. The amounts found in individual sheep varied widely.

TABLE 4.—Total amount of lead and arsenic eliminated by sheep and total found at autopsy in stomach and other organs

Sheep No.	Total lead arsenate fed	Total ingested		Total eliminated in—				Total recovered in—							
		Total ingested		Urine, As	Feces		Total, As	Stomach contents		Liver		Kidney		Heart	
		As	Pb		As	Pb		As	Pb	As	Pb	As	Pb	As	Pb
1	Grams 23.0	Grams 5.0	Grams 13.7	Milli-grams 49.28	Milli-grams 140.1	Milli-grams 1,695.2	Milli-grams 189.4	Milli-grams 263	Milli-grams 1,500.0	Milli-grams 4.5	Milli-grams 9.4	Milli-grams 1.8	Milli-grams 3.5	Milli-grams 0.11	Milli-grams 0.30
2	17.0	3.7	10.1	97.16	437.1	4,664.9	534.2	226	1,680.0	5.5	8.8			.22	.40
3	9.0	1.9	5.4	37.24	271.44	2,225.6	402.2	332	1,280.0	.73	3.1	.14	2.1	.16	.42
4	39.0	8.4	23.3	28.0	434.20	6,242.6		265	1,200.0	5.6	5.3	.75	.44		
5	6.0	1.29	3.57					576	2,040.0	10.6	10.8	2.5	2.9		
6	7.0	1.51	4.17												

† Apples included in ration.

TABLE 5.—Quantities of arsenic and lead found in lungs, bones, marrow, gall bladder, and wool of a sheep that was fed one-half of a gram of lead arsenate daily for 79 days

[Results expressed as milligrams per 100 gm.]

Element	Quantity in—					
	Lungs	Ribs	Femur	Marrow from femur	Gall bladder	Wool
As.....	<i>Milligrams</i> 0.38	<i>Milligrams</i> 1.42	<i>Milligrams</i> 0.35	<i>Milligrams</i> 1.11	<i>Milligrams</i> 0.01	<i>Milligrams</i> 0.28
Pb.....	3.0	2.5	.23	1.6	.15	.74

The findings at autopsy were similar to those described for the sheep in experiment 1. In those sheep that lived long enough for the phenomenon to be observed it was found that the erythrocyte count rose at first but fell to low levels a few days before death. In the sheep that lived 35 days, the count rose from approximately 8,000,000 when the feeding of lead arsenate began, to 13,000,000 on the tenth day thereafter, and then dropped to 4,200,000 on the twenty-seventh day. The leucocyte count fluctuated within normal limits until bacterial infection set in, when in most cases it rose sharply. A lead line was observed on the fourteenth day. On the twenty-sixth day a foul diarrhea appeared and the day before death hemoglobinuria was observed. The animal died in a cachexic state after 35 days.

On post mortem no erosions were found on the mouth, esophagus, or other portions of the digestive tract. A distinct lead line was observed. Enteritis was present to only a moderate extent. Fatty degeneration of the heart was evident and the lungs were congested and pneumonic. The spleen was ruptured and swollen, with the pulp distinctly darkened. The kidneys were blue black in color and had a metallic sheen. They were so soft that removal without breaking was difficult. The marrow of the flat bones was black. Upon exposure to the air the marrow became dark red. In the meat-packing industry animals showing this condition are known as "black cutters."

On post-mortem examination, lesions similar to those in sheep fed lethal amounts of lead arsenate were found in the sheep that received 0.25 g. of lead arsenate and died at the end of 35 days. The second sheep fed this amount continued to receive it for 94 days, when the lead arsenate feeding was discontinued. This sheep was in excellent condition when slaughtered 10 months later and showed no evidence of the earlier consumption of 23.5 g. of lead arsenate. It is of particular interest to note that when this animal was killed the bones showed no evidence of darkened marrow.

DISCUSSION AND SUMMARY

The toxicity of arsenic and lead appear to be affected by the nature and the solubility of the compounds in which they occur and the means by which they enter the body. Sheep were killed by the oral administration of 0.4 to 0.5 g. of arsenic (As) in the form of arsenic trioxide (As_2O_3), but substantially larger amounts of arsenic (As) in the form of lead arsenate were required to kill. When lead arsenate was sprayed on foliage and allowed to remain for several weeks, still larger amounts were necessary to cause death. A materially larger quantity of lead

arsenate was required to kill when the sheep consumed it on sprayed foliage than when it was administered experimentally in capsules. This should be further checked by carefully controlled experimental work involving the feeding of foliage carrying spray residue applied some weeks before the beginning of the experiments. If the experiments should show a change in the form of the lead and arsenic on foliage, together with a change in toxicity, this might explain the survival of animals that consumed large amounts of lead arsenate on sprayed foliage.

Nine sheep were fed lead arsenate varying in amount from 2 gm. per sheep per day to 0.25 gm. Apples were included in some of the rations. The animals receiving 1 and 2 gm. per day of lead arsenate died within a few days, in most cases after having received about 1.5 gm. of arsenic (2 gm. As_2O_3). One animal that received 0.25 gm. of lead arsenate per day survived for 35 days, and another that received the same amount for 94 days continued to live and was in good condition at the end of 10 months, when it was slaughtered. A total of 1.5 gm. of arsenic in the form of lead arsenate appears to be approximately a lethal dose when fed in small amounts daily. This is equivalent to about 41.0 mgm. of arsenic (As) or 192 mgm. of lead arsenate per kilogram of body weight.

Analytical results on the stomach contents and on a number of organs, as well as on the urine and feces, did not account for all the lead and arsenic consumed. The proportion of the arsenic consumed which was eliminated was rather small; the proportion of the lead eliminated was somewhat larger. The results obtained suggest a relation between the quantity consumed per day and the proportion eliminated. When larger quantities were consumed a larger proportion was eliminated in the urine; when smaller quantities were consumed a larger proportion was eliminated in the feces. Even in the latter case the quantity of lead was greater than that of arsenic. Paradoxically, the sheep that eliminated the smallest percentage of both arsenic and lead continued to live for 3 months. A larger proportion of lead than of arsenic was found in the stomach contents, presumably because of the difference in solubility. A larger proportion of the lead and arsenic remaining in the body was found in the stomach contents than elsewhere, although more was eliminated than remained in the stomach. The inclusion of apples in the ration had no apparent effect on the toxicity or elimination of lead and arsenic. Small quantities of these elements were found in the liver, kidney, heart, lungs, gall bladder, bones and marrow, and wool.

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COMPARISON OF A CHEMICAL AND A BIOCHEMICAL METHOD FOR DETERMINING THE BIOLOGICAL VALUE OF PROTEINS AND AN EVALUATION OF THE ENDOGENOUS NITROGEN¹

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INTRODUCTION

Since the discoveries that animals require specific amino acids and that amino acid distribution in proteins differs with a resultant difference in nutritive quality, a chemical method for determining the nutritive value of proteins has been sought. Almquist, Stokstad, and Halbrook (1)² proposed a chemical method which differed from any previously used. Analyses of animal protein concentrates were made for intact protein, protein decomposition products, indigestible protein, and hot-water-soluble protein. A formula was then used to give a numerical "protein quality index" (1, p. 205).

The purpose of the present study was to compare the method of Almquist et al. with the Mitchell and Carman (10) modification of the Thomas-Mitchell (8) nitrogen-retention method of determining the biological value of protein. The digestion of the protein-containing materials was compared *in vitro* and *in vivo*. In addition, a comparison was made between the endogenous urinary nitrogen output when corrected according to body weight and when corrected according to body surface area (12).

PROTEIN-CONTAINING MATERIALS

The protein-containing materials studied are given in table 1. The cereals and cereal protein concentrates were commercial products and were used without purification. Soybean meal 1 was a solvent-extracted meal at 60° C. that had been heated after extraction to 110° for 30 minutes to drive off the residual solvent. Soybean meal 2 was a portion of meal 1 that had been heated at 110° for 15 hours after extraction. It had the characteristic straw color of heated soybean meals. The dry whole-egg and liver meals were commercial preparations that had been soaked in alcohol, extracted with ether for 48 hours and air-dried. The tankage, a commercial meat and bone product having about 50 percent of crude protein, was soaked in 95-percent ethanol, extracted with ethyl ether for 48 hours, and air-dried. The casein was a commercial casein which was purified twice by dissolving in ammonia, running through a supercentrifuge, and reprecipitating with a mixture of acetic and hydrochloric acids; it was then extracted with alcohol for 24 hours, with ether for 48 hours,

¹ Received for publication October 4, 1939. Paper No. 1681, Scientific Journal Series, Minnesota Agricultural Experiment Station. The data in this paper are taken from a thesis presented by Floyd C. Olson in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota, 1938. The original data are on file in the Division of Biochemistry, University of Minnesota.

² Italic numbers in parentheses refer to Literature Cited, p. 341.

and dried at 40° in vacuum. The crude-protein content of the prepared foods is given in table 1.

TABLE 1.—*True digestibility and biological value of proteins tested by the nitrogen-retention method*

Source of protein	Crude pro- tein con- tent as pre- pared	Propor- tion in diet	Tests	True digestibility		Biological value	
				Average with standard error	Stand- ard devia- tion	Average with standard error	Stand- ard devia- tion
	<i>Percent</i>	<i>Percent</i>	<i>Number</i>	<i>Percent</i>		<i>Percent</i>	
Dried whole egg.....	77.18	8	12	97.9±0.17	0.58	93.9±0.82	2.84
Casein.....	91.68	8	11	99.7±.35	1.16	62.9±1.83	6.09
Whole wheat.....	13.40	8	11	91.7±.59	1.97	47.2±1.28	4.24
Wheat gluten.....	76.38	8	10	99.3±.21	.66	41.9±1.44	4.56
Whole corn.....	10.06	8	12	91.3±.63	1.83	52.5±1.10	3.80
Corn-gluten meal.....	42.63	8	10	96.7±.41	1.30	41.8±1.71	5.40
Liver meal.....	76.25	8	12	88.3±.40	1.38	56.5±.84	2.90
Tankage.....	56.19	12	14	83.3±.50	1.84	38.1±1.63	6.10
Soybean meal 1.....	47.70	8	10	83.9±1.10	3.48	61.1±1.16	3.68
Soybean meal 2.....	46.00	8	10	84.3±.86	2.70	68.5±1.06	3.35

EXPERIMENTAL METHODS

NITROGEN BALANCES

Two different series of nitrogen-balance experiments were run. The first series was performed in the Division of Agricultural Biochemistry, University of Minnesota. The metabolism cages and collection of excreta were essentially as described by Mason and Palmer (7). To prevent loss of scattered feed a porcelain feed dish was set into the center of a 1-pound coffee can, the side of which was cut down in such a way that wire netting placed over the top gave an incline of about 40°.

The biological values of the tankage and soybean meals 1 and 2 were determined in the Department of Agricultural Chemistry at Ohio State University, where a different technique was necessary because of a different type of cage. The technique used here was essentially that of Mitchell (8) except that the filter paper containing the absorbed urine was submitted to direct nitrogen determination, blanks being run on the same amount of similar filter paper.

The experimental protein rations furnished 4.5 percent of dry whole-egg protein in the nitrogen-free periods, and 8 percent of protein in the protein-feeding periods, except for the tankage which was fed at a 12-percent level in order to promote growth. To this was added 4.5 percent of Hawk and Oser (5) salt mixture, 1 percent of NaCl, 2 percent of cod-liver oil, filtered butterfat to give a total fat content of 10 percent, agar to give a crude-fiber content of about 2 percent, 0.3 percent of vitamin B₁ concentrate,³ and tapioca dextrin to complete the 100 percent.

In the first series young male rats only were used from the highly inbred strains in the rat colony of the Division of Biochemistry, University of Minnesota. In the second series the rats, including eight females, were purchased from a commercial breeder.

All the experiments involved 4-day transition periods and 7-day collection periods. Two experimental protein-containing rations were

³ A commercial product, containing 105 International Units of vitamin B₁ per gram and 9.04 percent of nitrogen.

fed in the periods between initial and final nitrogen-free periods. The rats were so divided that one-half the products were tested in the first protein-feeding period and one-half in the second.

The appetite of the rats during the nitrogen-free periods was good, and they all gained slightly in weight. The food intake on the wheat gluten and corn-gluten meal was rather low, but all rats which did not gain weight were discarded.

CHEMICAL ANALYSES

The same protein products used in the feeding experiments were analyzed by the procedure of Almquist and associates (1). In this method pepsin HCl alone is the digesting agent. In order to test its completeness, peptic⁴ digestion was followed by tryptic,⁵ the solution being made alkaline to an alkalinity of 0.5-percent sodium carbonate with solid sodium carbonate, 25 ml. of 1.5-percent trypsin solution was added, and the sample shaken by machine for another 24 hours at 40°C. The insoluble material was analyzed for nitrogen. Blanks for the enzymatic digestion were run in the same manner as when protein was present. The activity of the enzyme preparations is indicated by the fact that they effected nearly 100-percent digestion of casein.

It was noticed in the peptic-tryptic digestion of liver meal and tankage that a copious reddish-brown precipitate formed on neutralizing with sodium carbonate. This was thought to be porphyrin, the hydrochlorides of which are soluble but which are insoluble in neutral or basic solution. A nitrogen analysis of the porphyrin precipitate was made in separate experiments to determine how much of this fraction classed as digestible by the procedure of Almquist et al. (1) would be classed as indigestible in the pepsin-trypsin digestion.

EXPERIMENTAL DATA

The endogenous urinary nitrogens in the nitrogen-balance experiments, corrected for body weight, are presented graphically in figure 1. The values used for plotting figures 1 and 2 were taken from table 2 and a third *N*-free period on 11 rats not shown in this table. The formula for the regression line was first calculated in terms of logarithms and then converted into arithmetical terms. The formula is $N = 135.6 W^{-0.469}$, where *N* equals endogenous urinary nitrogen in milligrams and *W* equals body weight in grams. The negative slope of the curve indicates that the endogenous urinary nitrogen per unit of body weight decreases with an increase in body weight. This formula differs considerably from Ashworth's (3) formula, $N = 69.3 W^{-0.274}$ calculated for certain of Mitchell's data on young rats.

Smuts (12) reported that the endogeneous urinary nitrogen is more closely related to body surface than to body weight. Figure 2 shows the same data as figure 1 plotted in relation to the body surface, this being calculated by Lee's (6) formula, $S = 12.54 W^{0.60}$ where *S* is the body area in square centimeters and *W* is body weight in grams. The formula for the regression line in figure 2 is $N = 5.61 A^{0.069}$, where *N* is the endogenous nitrogen in milligrams and *A* is body-surface area in square centimeters. When the data are calculated according to Fisher's (4) *t*-value, the exponent is shown to be not significantly different from zero.

⁴ Powdered pepsin, U. S. P. X., Merck, was employed. ⁵ Trypsin, Difco, 1:110, was employed.

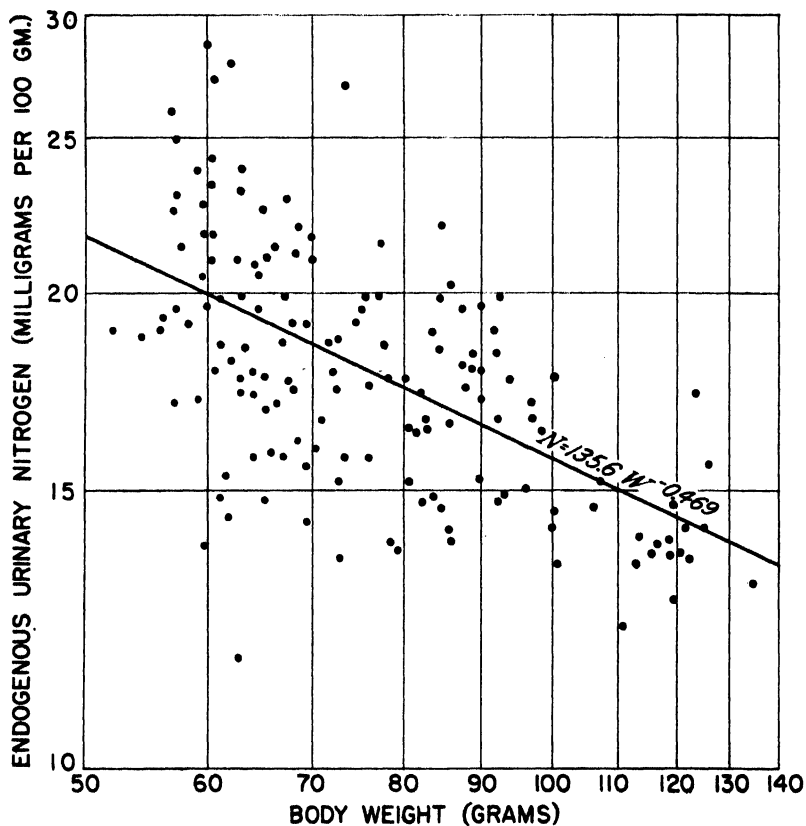


FIGURE 1.—Regression curve of milligrams of endogenous urinary nitrogen per 100 gm. of body weight plotted against body weight.

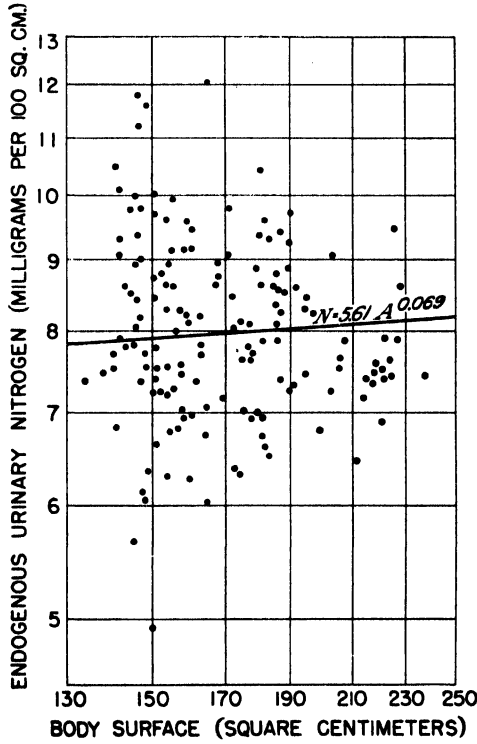


FIGURE 2.—Regression curve of milligrams of endogenous urinary nitrogen per 100 cm.² of body surface plotted against body-surface area.

TABLE 2.—Summary of endogenous urinary nitrogen data from which figures 1 and 2 were drawn

Rat No.	First N-free period				Second N-free period			
	Average weight of rat	Food consumed daily	Endogenous urinary N daily	Endogenous urinary N per 100 cm. ² of body surface	Average weight of rat	Food consumed daily	Endogenous urinary N daily	Endogenous urinary N per 100 cm. ² of body surface
	Grams	Grams	Milli-grams	Milli-grams	Grams	Grams	Milli-grams	Milli-grams
1	89	7.6	15.2	9.55	125	10.5	17.9	7.88
2	85	7.4	14.7	9.58	127	10.6	19.8	8.63
3	85	7.1	11.5	7.49	124	10.9	21.4	9.46
4	54.5	5.9	10.3	7.46	106.5	8.1	15.7	7.61
5	67	6.7	12.5	8.00	103.5	6.3	14.7	7.24
6	66.5	6.6	14.2	9.13	106	6.1	15.5	7.53
7	61.5	6.4	9.0	6.06	92.5	5.9	18.5	9.75
8	61	6.3	9.1	6.16	104.5	6.1	18.4	9.02
9	65.5	6.8	9.7	6.29	81	5.1	12.3	7.02
10	71	7.3	13.3	8.22	119.5	8.2	15.3	6.91
12	61.5	6.8	9.5	6.40	108	8.1	16.4	7.88
13	65	6.8	12.7	8.27	86	6.2	12.2	6.72
14	67.5	8.2	13.5	8.60	87.5	8.0	17.1	9.32
15	63	7.2	12.7	8.43	81	7.0	13.3	7.60
16	64.5	7.7	13.5	8.84	83	6.9	13.8	7.77
17	65.5	8.3	13.8	8.95	88.5	8.9	15.4	8.34
18	60.5	7.3	13.2	8.98	72.5	6.8	12.6	7.69
19	58	6.7	12.4	8.65	78.5	8.0	13.8	8.03
20	68	7.4	11.8	7.48	120.5	9.6	16.5	7.42
21	68.5	7.3	11.1	7.01	86	6.0	12.0	6.61
22	62.5	6.7	13.1	8.74	73.5	5.5	11.6	7.02
23	59	5.8	12.4	8.56	70	4.9	11.2	6.98
24	64	7.3	11.0	7.24	79	5.0	11.0	6.38
25	64	7.5	10.1	6.64	111	8.1	13.7	6.47
26	77.5	8.3	15.4	9.03	96.5	5.6	14.5	7.46
27	75	8.0	14.4	8.61	92.5	6.8	13.7	7.22
28	65.5	7.5	11.1	7.20	80	5.0	11.0	6.35
29	69.5	6.7	11.1	6.95	83	5.2	12.3	6.82
30	78.5	8.2	14.6	8.49	98.5	7.6	16.2	8.25
31	60.5	6.1	10.8	7.35	73	4.0	9.9	6.02
32	62	7.4	11.2	7.51	83	8.5	13.7	7.71
33	61.5	6.7	11.4	7.68	75.5	6.2	14.7	8.76
34	58.5	6.0	11.2	7.78	76.5	7.0	12.1	7.15
35	52	6.3	9.9	7.38	67	6.0	10.6	6.78
36	57.5	6.0	11.8	7.92	72	6.3	12.8	7.84
37	57.5	6.2	13.3	9.33	70	6.8	15.2	9.47
38	57.5	6.1	14.4	10.10	69.5	5.7	10.0	6.26
39	71	7.4	11.9	7.35	82.5	5.8	14.3	8.07
40	63	5.9	10.9	7.24	68.5	4.8	14.5	9.15
41	73	7.4	11.1	6.75	81.5	6.1	13.4	7.62
42	66.5	6.3	11.3	7.26	70	4.4	14.7	9.16
43	56.5	6.3	10.9	7.73	69.5	5.3	13.3	8.32
44	63	6.9	11.1	7.37	73.5	6.1	19.9	12.05
45	66	7.4	10.5	6.78	80	6.2	8.11	8.11
46	60	5.9	11.8	8.07	67.5	5.3	15.5	9.87
47	56	6.4	10.6	7.55	68	6.1	11.9	7.55
48	68	6.7	13.0	8.24	78	5.6	16.8	9.81
49	63.5	6.6	11.8	7.80	72	5.5	13.4	8.21
50	63	8.0	(¹)	(¹)	93	7.7	13.9	7.30
51	60	7.8	8.3	5.67	97	8.0	16.5	8.46
52	61	8.0	12.1	8.19	97	8.0	16.2	8.30
53	61	7.9	14.3	9.68	89	8.0	15.0	8.26
54	63	8.0	7.4	4.94	92	8.0	17.5	9.26
55	57	8.0	9.7	6.84	85	7.4	15.6	8.65
56	63	8.0	(¹)	(¹)	84	7.9	12.5	6.98
57	59	8.0	11.3	7.80	86	8.0	17.5	9.64
58	60	7.9	12.3	8.41	85	7.9	16.9	9.37
59	62	7.9	17.3	11.60	76	6.8	13.3	7.89
60	59	7.9	(¹)	(¹)	93	7.9	15.4	8.09
61	60	7.9	13.7	9.36	89	8.0	16.3	8.80
62	60	7.9	13.1	8.95	90	7.9	15.4	8.25
63	65	7.8	13.3	8.66	94	8.0	16.5	8.62
64	64	7.9	11.4	7.50	92	8.0	16.8	8.89
65	63	8.0	14.6	9.69	85	8.0	18.8	10.43
66	63	8.0	15.1	10.02	90	7.9	16.0	8.67
67	60	7.8	14.6	9.98	76	7.5	15.1	8.96
68	62	8.0	(¹)	(¹)	90	8.0	17.6	9.43
71	60	7.9	17.2	11.76	90	7.7	13.8	7.40
72	59	7.7	14.1	9.74	86	7.7	14.3	7.87
73	60.5	7.9	16.5	11.22	88	7.7	15.8	8.68
74	57	7.7	14.9	10.50	84	7.7	15.9	8.88
75	57	7.9	12.8	9.02	85	7.7	12.5	6.93

¹ First endogenous urinary N lost. Results based on second period.

When the data from Mitchell's laboratory, as used by Ashworth (3) are plotted in figure 3 on the body-surface-area basis, the regression formula $N = 3.44 A^{0.200}$ is obtained. This formula shows a larger increase in endogenous urinary nitrogen per unit of body surface with an increase in body surface than does the regression line in figure 2. An inspection of figure 2 shows a fair grouping of the values around the regression line. However, the values from Mitchell's laboratory show no grouping around the regression line but a wide scattering over the range of 5 to 17 mgm. per 100 cm.² of body surface. This wide scattering of the points appears from the original data to be owing more to a variation between groups on different rations than to differences between individual animals. This would indicate that the variation was due to lack of complete control of the experimental procedure. For example, in some cases, even with whole egg in the ration, the animals lost weight, indicating that the food intake was not sufficient for the energy requirements. This would tend to increase the endogenous urinary nitrogen. Ashworth (2) reported that a 4-day preliminary period on a nitrogen-free ration is not sufficient for attainment of a low food intake. Mitchell and Beadles (9) reported a case in which the true endogenous level was not reached with a 4-day preliminary period.

However, it is to be noted that some of the values from Mitchell's data are for rats larger than those plotted in figure 2. Other determinations of endogenous urinary nitrogen on adult rats (not reported here) have given values considerably higher than 8 mgm. per 100 cm.² of body surface even though a longer depletion period on a nitrogen-free ration was used. Smuts (12) found that rats weighing 150 gm. or more give an average endogenous urinary nitrogen of 15.40 mg. daily per 100 cm.² of body surface. Calculations made from results obtained by Mason and Palmer (7) and by Lohn⁶ from this laboratory on adult rats show values almost as high as those obtained by Smuts. Thus, it would seem that, whereas young rats of 50 to 100 gm. body weight show an endogenous urinary nitrogen output of 6 to 10 mg. per 100 cm.² of body surface, older rats attain a considerably greater average output when calculated per unit body surface.

Table 1 gives the biological value and true digestibility with the standard errors and standard deviations for the test proteins. For the biological value calculations, the endogenous urinary nitrogen was corrected for changes in body surface area, and the metabolic fecal nitrogen was corrected for each gram of dry food intake.

Table 3 shows the significance of the differences between certain means, by using formulas given by Treloar⁷ and referring to Shepard's (11) tables for the relative deviate, x' , in the determination of the probability for comparisons of biological value and digestibility. For comparisons of endogenous urinary nitrogen where correlation is expected, Fisher's (4) formula was used.

⁶ LOHN, C. A STUDY OF THE EFFICIENCY OF FOOD METABOLISM FOR THE MAINTENANCE OF INBRED ANIMALS DIFFERING IN THEIR EFFICIENCY OF FOOD UTILIZATION DURING GROWTH. Thesis, Ph. D., Univ. Minn.

⁷ TRELOAR, ALAN E. AN OUTLINE OF BIOMETRIC ANALYSIS. 3 v. in 1. Burgess Publishing Co., Minneapolis. 1935. [Mimeographed.] See pp. 28-29, and 56.

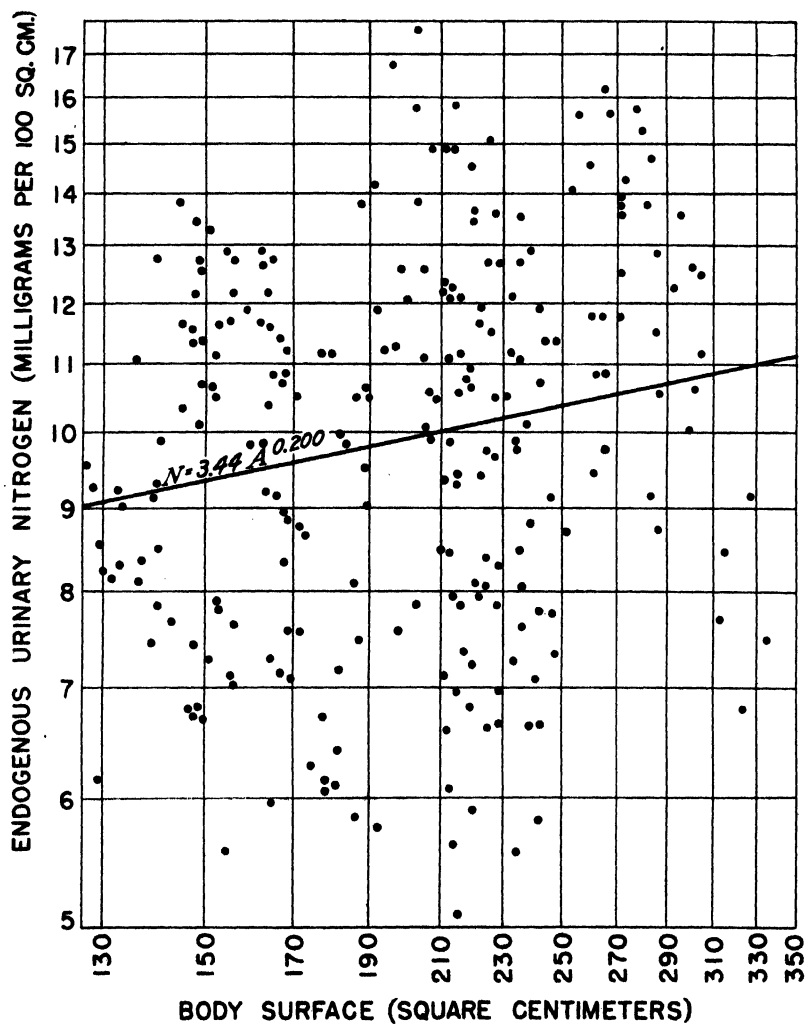


FIGURE 3.—Regression curve of milligrams of endogenous urinary nitrogen per 100 cm.² of body surface plotted against body-surface area. Mitchell's data as cited by Ashworth (3).

TABLE 3.—*Determination of significance of differences in means for endogenous urinary-nitrogen and other data*

Item	Period or value	Mean	Standard deviation	Probability
		<i>Milligrams</i>		
Endogenous urinary nitrogen per 100 gm. of body weight.	First N-free period	19.6	3.4	0.001
	Second N-free period	17.1	2.6	
Endogenous urinary nitrogen per 100 cm. ² body surface.	First N-free period	8.2	1.4	.674
	Second N-free period	8.1	1.1	
Endogenous urinary nitrogen per 100 gm. of body weight, Mitchell's data (3).	First N-free period	22.1	5.0	.001
	Second N-free period	18.8	6.1	
Endogenous urinary nitrogen per 100 cm. ² of body surface, Mitchell's data (3).	First N-free period	10.2	2.2	.92
	Second N-free period	10.3	3.3	
		<i>Percent</i>		
Whole wheat	Biological value	47.2	4.2	.006
Wheat gluten	do.	41.9	4.6	
Whole corn	do.	52.5	3.8	.001
Corn-gluten meal	do.	41.8	5.4	
Soybean meal 1	do.	61.1	3.7	.001
Soybean meal 2	do.	68.5	3.3	
Soybean meal 1	Digestibility	83.9	3.5	.83
Soybean meal 2	do.	84.3	2.7	

Table 3 shows that when the endogenous urinary nitrogen is corrected for body weight, a significant fall is noted between the first and second periods, but not when the correction is made for body surface. This is also shown to be true for the data from Mitchell's laboratory (3). A significant difference is shown between the mean biological value of whole-wheat and wheat-gluten proteins, between whole-corn and corn-gluten proteins, and between raw and heated soybean-meal proteins. However, no significant difference is shown in the digestibility of the two soybean meals.

The results obtained by the chemical method of Almquist et al. (1) are given in table 4. The digestibility determinations of the proteins in vitro do not agree very closely with the corrected digestibility obtained from the rat data. The corn proteins show a higher digestibility in vivo while whole-wheat, soybean, and tankage proteins tend to give better digestion in vitro. The differences between digestion of tankages and liver meal by pepsin HCl and on peptic-tryptic digestion show that a considerable amount of porphyrin-like material is present, and therefore there is a distinct error in the method employed by Almquist and associates for determining digestibility. The digestibility used in calculating the protein quality index was the value obtained in vitro which most nearly approached the digestibility in vivo. The explanation of this is given in the last column of table 4. The data indicate that no single method of enzymatic digestion is comparable with animal digestion.

TABLE 4.—Results obtained for digestibility of proteins from various sources, expressed as percentage of total nitrogen, chemical (*in vitro*) means of digestion and determination being used

Source of protein	Copper precipitable (A) ¹	Phosphotungstic acid precipitable (D) ¹	Undigested with pepsin (B) ¹	Undigested with pepsin neutralized after digestion	Undigested with pepsin and trypsin	Undigested residue as determined from rat data	Hot-water soluble (C)	Protein quality index	Method of digestion used for calculation
Dried whole egg	100.0	0.0	4.2	-----	0.0	2.2	4.1	93.3	Pepsin.
Casein	95.7	.5	4.7	-----	.1	.3	3.8	93.5	Trypsin.
Whole wheat	81.3	4.2	5.8	-----	3.5	8.3	13.4	69.2	Pepsin.
Wheat gluten	84.0	8.3	.6	-----	.2	.7	16.0	77.1	Do.
Whole corn	85.4	8.7	41.0	-----	17.4	8.7	2.0	70.3	Trypsin.
Corn-gluten meal	94.0	2.3	10.8	-----	5.9	3.3	1.8	87.9	Do.
Liver meal	86.9	5.9	11.0	15.3	11.6	11.7	11.5	73.1	Do.
Tankage	78.9	11.7	11.1	13.9	9.0	16.7	16.2	60.0	Pepsin, neutralized.
Soybean meal 1	95.4	.3	9.9	-----	3.5	16.1	9.0	80.1	Pepsin.
Soybean meal 2	96.2	.4	11.3	-----	3.7	15.7	6.5	81.2	Do.

¹ Protein quality index = $A - (B + 0.6 C) + 0.4 D$.

The data of tables 1 and 4 are compared in figure 4. There seems to be little correlation between the two methods. However, it will be noted that the natural foods, to which no protein material had been added or from which none had been subtracted, lie on a fairly straight line. Casein, corn gluten, and wheat gluten, which are more or less isolated proteins, give quite different results by the chemical method as compared with the nitrogen-retention method.

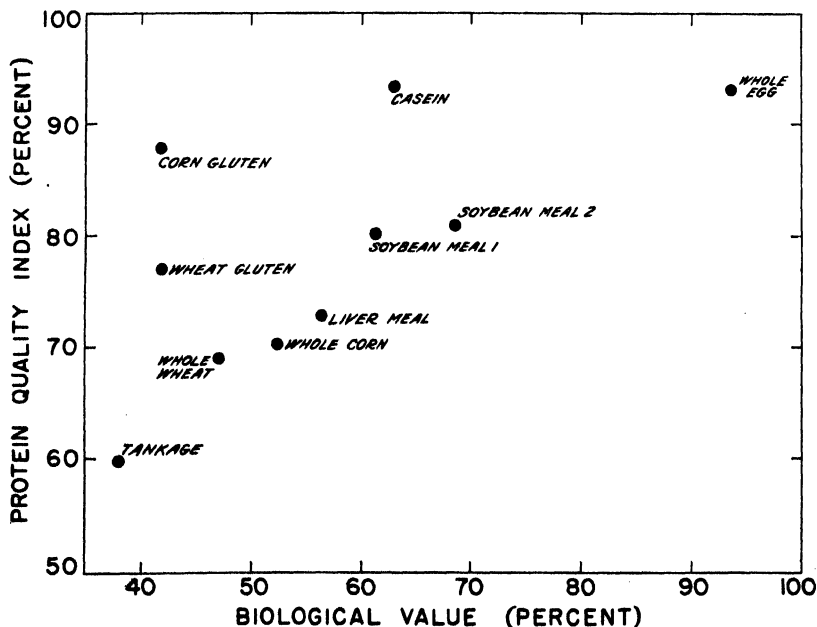


FIGURE 4.—Comparison of the protein quality index with the biological value for proteins from different sources. Data from tables 1 and 4.

SUMMARY

Nitrogen-balance studies were made with young rats, 10 food proteins being used. The values obtained (rounded) were: Whole egg 94, casein 63, whole wheat 47, wheat gluten 42, whole corn 52, corn-gluten meal 42, liver meal 56, meat and bone tankage 38, solvent-extracted soybean meal 61, and heated solvent-extracted soybean meal 68.

The same foods were used for the determination of the protein quality index by the Almquist chemical method.

The digestibility of the proteins was calculated from data obtained by the nitrogen-retention method. An attempt was made to compare these values with the digestibility determined by means of enzymes. The digestibility *in vitro* showed a poor comparison with the digestibility *in vivo*.

The protein quality index showed no comparison with the biological value by the nitrogen-retention method when used on isolated proteins. For natural foods the comparison is fair if the enzymic digestibility most similar to that *in vivo* is used for the calculation of the protein quality index.

Heated soybean meal was found to have a higher biological value than raw, but no difference was found in the digestibility of the raw and heated meals.

Correction of the endogenous urinary nitrogen according to body surface was found to give less variation than correction according to body weight. When the body surface correction was made, no difference was shown in endogenous urinary nitrogen between the first and second nitrogen-free periods.

The regression lines were plotted for the endogenous urinary nitrogen when calculated according to body weight and body surface area. The formula for the regression line for the body weight graph is $N = 135.6W^{-0.469}$, and for the body surface graph, $N = 5.61A^{0.068}$.

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COMPARISON OF THE ACCURACY OF TWO METHODS OF ESTIMATING FINENESS OF WOOL FIBERS¹

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INTRODUCTION

Diameter of wool fibers is one of the most important points to be considered in evaluating a fleece, either with respect to some phase of scientific work or from a commercial viewpoint. The method most commonly used for estimating the diameter is to compare fibers from a sample in question with standard fibers of known diameter or with a mental picture of such standards. Hardy and Wolf (4)² recently described a method in which enlarged cross sections of the fibers in question were compared with similarly enlarged fibers of known diameters which make up the set of standards. The object of the present study was to compare the accuracy of these two methods.

MATERIAL AND METHODS

One hundred samples of wool were selected from samples available in the laboratory. These were obtained from Rambouillet, Corriedale, and Columbia yearling ewes at the United States Sheep Experiment Station, Dubois, Idaho. The average diameter of each sample was determined by measuring the actual diameter of the fibers, up to a maximum of 100, in a given area of the cross section. In some of the coarser samples there were not 100 fibers in the area; in 17 samples there were only 50 fibers, and in 13 samples 90 fibers. The diameter of each fiber was obtained by taking 2 measurements at right angles to each other and obtaining the average of these 2 measurements. These determinations were made after completion of the scoring work on the 100 samples. Consequently, there was no information on actual diameters available to the judges at the time the scoring was done.

As a check on the actual diameters of the fibers, the diameters were estimated by the count method described by Hardy and Wolf (4). A correlation of 0.98 was found between diameters estimated in this manner and the direct measurements of fibers described.

For convenience, the two methods compared in this study are designated in this paper as method A, in which enlarged cross sections of fibers were compared with cross sections of known diameter, and method B, in which fibers were compared directly with those of known diameter.

In method A, a bundle of fibers from each sample was cross-sectioned by the method described by Hardy (3). The cross sections were photographed on positive 35-mm. film strips and projected to 1,000 magnifications for comparison with the standard, which consisted of a

¹ Received for publication November 3, 1939.

² Italic numbers in parentheses refer to Literature Cited, p. 349.

similar film having photographs of cross sections of known diameter magnified to the same extent. The various cross sections in the standard set were projected in succession adjacent to the image of the unknown until the observer decided which sample of known diameter the unknown most nearly approached. This method is described in detail by Hardy and Wolf (4). The standard set used contained the following nine wool grades, based on spinning counts: 80s, 70s, 64s, 60s, 56s, 52s, 48s, 44s, and 40s.

In method B, fibers from the samples were compared with fibers in a set of standard samples, which included the same nine grades as were used in method A. This set of samples was selected as being representative of the respective grades on the basis of both visual appearance and measurements of the diameter of cross sections.

The diameter of each sample was estimated by each method on 3 different days by 5 judges all of whom were experienced in the grading of wool. Thus 1,500 observations were made by method A, and the same number by method B. Statistical analysis of the data was made to determine the accuracy of each method. The statistical procedures are indicated in the discussion of each phase of the analysis. For convenience in analysis and in presentation, the grades arrived at in both methods were classified from 1 to 9, corresponding to the wool grades of 80s to 40s. The term "score" as used subsequently in this paper refers to the class numbers representing the grades into which the samples were classified by the judges.

EXPERIMENTAL RESULTS

DIFFERENCE IN SCORING LEVELS OF THE TWO METHODS

The placement of the samples by the five judges, in methods A and B, is given in table 1. The distribution of the samples based on actual measurements of cross sections is also given in table 1 for comparison with the scores given by the judges. The superiority of method A as evaluated by this procedure is obvious, 96 percent of the samples being placed in the correct class or deviating only 1 class from the correct one, whereas with method B only 44 percent were placed correctly or within 1 class of the correct one.

In the greater number of deviations from the actual values under method A the diameters of the fibers were underestimated, that is, they were estimated to be finer than they actually were; whereas in method B the diameters of the greater number were overestimated that is, estimated to be coarser than they were. Since the totals given in table 1 are based on 100 samples and are average placements of 5 judges, the numbers may be considered as percentages or probability placements.

Another method of showing the accuracy of the determinations by the use of methods A and B is given in figure 1. The frequency based on actual measurements is plotted against the class scale and is given by the solid line. The placement of samples according to method A follows closely that of the direct measurements, whereas the distribution by the use of method B is strikingly different and indicates that the judges, not being able to distinguish the relative diameters of the fibers, tended to place them toward the middle of the scale.

TABLE 1.—Comparison of methods A and B in average placement of 100 wool samples by 5 judges ¹

Class No.	Range in average diameter of fibers ²	Samples in each class—													
		As found by actual measurements	Deviating from actual measurements by number of classes indicated (+ signifies overestimation; —, underestimation), as estimated by—												
			Method A					Method B							
			0	+1	—1	+2	—2	0	+1	—1	+2	—2	+3	—3	+4
	Microns	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
1	16-17.9	11	8	3	—	—	—	—	—	—	6	—	1	—	4
2	18-19.9	16	6	2	8	—	—	2	2	—	2	—	10	—	—
3	20-21.9	27	14	4	9	—	—	3	—	2	18	—	2	—	1
4	22-23.9	21	10	2	9	—	—	1	14	—	5	—	—	—	—
5	24-25.9	8	5	1	2	—	—	3	3	—	—	—	1	—	—
6	26-27.9	6	1	—	5	—	—	—	—	2	2	—	—	—	—
7	28-29.9	4	1	—	2	—	1	2	2	—	2	—	—	—	—
8	30-31.9	5	—	—	2	—	—	2	2	—	—	1	—	—	—
9	32-37.9	2	—	—	2	—	—	—	—	2	—	—	—	—	—
Total		100	45	12	39	0	4	13	25	6	34	1	15	0	6

¹ For description of methods, see p. 343 and 344.

² The range in diameter in each class was arrived at arbitrarily, the distribution curve being based on actual measurements of the ninth class, which is wider because of the few samples occurring in this group; therefore, the ranges differ somewhat from those given by Hardy and Wolf (4).

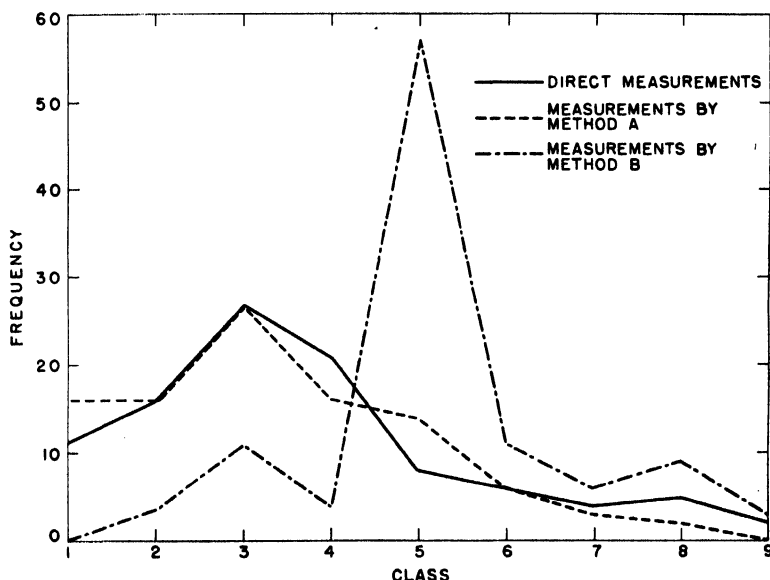


FIGURE 1.—Distributions of average diameters of 100 samples of wool, as determined by 3 different methods.

The frequencies obtained with methods A and B were correlated with the frequency obtained with the direct measurements. In method A, the correlation was 0.95; in method B, 0.77. These correlations are significantly different from a population in which the correlation is zero. In order to test the difference between the corre-

lations of 0.95 and 0.77, the method given by Fisher (2) was used. The probability of these values arising from the same population is less than 0.001. This finding is taken as statistical support to the conclusion that methods A and B are completely different in their placements of the samples in relation to the standard values.

SOURCES OF VARIANCE IN THE TWO METHODS

In order to evaluate the elements that contribute to the variability in the procedures, the data obtained by each method, A and B, were studied separately by using Fisher's analysis of variance (2). The mean squares calculated in that analysis were then transformed into percentages according to a plan first adopted by Lush³ in a similar study, and later used in a study of two scoring methods employed in evaluating certain swine characters by Hetzer and Phillips (5). Table 2 gives the proportion of the variance due to factors which it is believed have an important influence on the variability of the scores. The method for testing significance was the *F* test given by Snedecor (6) for differences between mean squares as calculated in the analysis.

TABLE 2.—Percentage of variance contributed by each source to the total variance in the scores by the 2 methods

Source of variance	Method A	Method B	Source of variance	Method A	Method B
	<i>Percent</i>	<i>Percent</i>		<i>Percent</i>	<i>Percent</i>
Between samples.....	¹ 82.31	¹ 70.73	Judges×days.....	² 0.59	² 0.69
Between judges.....	¹ 4.76	¹ 4.56	Samples×judges×days ..	9.56	4.58
Between days.....	1.46	.03			
Judges×samples.....	2.53	¹ 13.02	Total.....	100.00	100.00
Days×samples.....	— .21	6.40			

¹ Highly significant ($p < 0.01$).

² Significant ($p < 0.05$).

The error term used in testing the significance was the source of variance listed as samples×judges×days. The percentages of variance owing to this source are included in table 2 to show the relative importance of causes of variation other than the first six listed. A part of the variance expressed by this error term evidently is caused by random error in scoring; however, other factors such as shifts in individual opinion of the judges in scoring may also be important causes of this variance.

In both methods the variance due to differences between samples was highly significant. Since the methods are designed to measure differences between samples, the one that demonstrates the greater percentage of variance in this item would be the better method for detecting differences.

The variance due to differences between judges was sufficiently large to indicate highly significant differences in the scoring levels of the judges. The amount of variation contributed by this source was about equal in the two methods.

Variance due to differences between days was small in both methods but is sufficiently large to be highly significant in method A because it enters into the scoring 500 times a day; therefore, the mean square becomes significantly large.

³ LUSH, JAY L. REPEATABILITY OF SCORES MADE BY THE SAME MAN. [Unpublished.]

Of the double interactions, only one, the interaction of judges and samples in method B, was highly significant. Interactions of judges and days were significant in both methods, although in neither case is the variation large enough to be considered important.

CORRELATIONS BETWEEN SCORES

The extent to which the scores of the samples were likely to be the same on different days, the correspondence of the scores of the same judge on different days, and the extent to which the judges agreed in their scores for the same sample, are given by the intraclass correlations in table 3. These correlations are all highly significant. The correlations are similar in size for both methods, but in all cases those for method A are slightly higher, indicating a tendency for greater accuracy of method A as judged by these intraclass correlations.

TABLE 3.—*Intraclass correlation between the scores given each sample by the 2 methods*

Intraclass correlation	Method A	Method B
Between scores of samples on different days.....	0.98	0.90
Between scores of the same judge on different days.....	.87	.86
Between scores of the same sample by different judges.....	.93	.82

INDIVIDUAL-JUDGE PLACEMENTS

Since it is of interest to know how the individual judges placed the scores in the two methods, the data were grouped by judges, as given in table 4. The number of times each judge placed the sample correctly is given under each method. The plus and minus deviations also are given. The correlations (r) indicate the consistency of the judges in placing the samples. For example, judge No. 5 under method A placed the fewest samples correctly. However, the consistency with which he placed the samples is shown by the highest correlation, 0.84. Under method B the judges seemed to be consistent in their placement as indicated by the correlations, but the number of accurate placements was low, and the deviations were preponderately on the plus side, that is, the fibers were judged to be coarser than they actually were.

TABLE 4.—*Placement of scores by the individual judges, using the 2 methods*

Judge No.	Deviation from actual measurements by number of classes indicated (+ signifies overestimation; —, underestimation) as estimated by—																	
	Method A								Method B									
	—3	—2	—1	0	+1	+2	+3	r	—2	—1	0	+1	+2	+3	+4	+5	r	
1.....	No.	No.	No.	No.	No.	No.	No.	0.81	No.	No.	No.	No.	No.	No.	No.	No.	0.61	
2.....	6	28	48	18	1	7	---	.81	3	7	11	25	24	22	9	2	.63	
3.....	1	12	47	33	9	---	---	.82	3	6	13	30	30	11	4	3	.79	
4.....	7	39	44	9	---	---	---	.81	2	8	27	27	25	10	1	---	.61	
5.....	7	38	43	10	1	1	---	.84	2	8	6	24	22	24	12	2	.67	
6.....	17	44	37	2	---	---	---	---	1	---	15	27	25	24	6	2	---	

The general observations to be made from table 4 are that all judges were working within either method with approximately the same degree of success, and the relative accuracy of the two methods is not biased by individual judges.

DISCUSSION

The experimental findings indicate clearly that method A is more accurate in estimating wool-fiber diameter than is method B. With method A the judge not only is able to see the cross sections of fibers at 1,000 magnifications, but also he can view them without being confused by grease and dirt, crimp, and differences in light refraction. Furthermore, he is able to note and consider variability in the diameters of individual fibers. The psychological and physiological effects of these various factors on the wool scorer are discussed by Tänzer (?).

The fundamental basis of the visual judgment of the fineness of Wool is the Fechner-Weber psycho-physical law, as discussed by Barker (*l. pp. 141-151*). This law was stated by Fechner in 1860 as follows: "In order that the intensity of a sensation may increase in arithmetical progression the stimulus must increase in geometrical progression." His general formula is $I=C \log S$, where I is the sensation, S the stimulus, and C a constant. The failure of the judge to place the fineness of wool in the correct groupings or classes seems to indicate that the so-called law does not hold.

Method A requires much more equipment and time than method B, and in some types of work this expenditure would not be justified. However, it seems evident that method B cannot be expected to yield satisfactory data for experimental or other purposes in cases in which the detection of small differences in diameter is essential.

SUMMARY

A comparison is made of the accuracy of two methods for estimating the diameters of wool fibers. In method A the diameters of cross sections of 100 wool samples, after being projected to 1,000 diameters, were estimated by comparing with a standard set of known diameters enlarged to the same magnitude. In method B the diameters of wool fibers from the same 100 samples were estimated by comparing with a standard set of wool samples of known diameters. Five judges estimated the diameter of each of the 100 samples on three different days, making a total of 1,500 observations by each method. For comparison, the average diameters of the 100 samples were determined by actual measurements of the fibers in enlarged cross sections.

By method A, 45 of the 100 samples were placed correctly, based on the average scores for the judges, and only 4 deviated as much as 2 classes from the actual measurements. By method B only 13 samples were placed correctly, and 6 samples deviated 4 classes from the classes in which they were placed by direct measurements. Judges tended to underestimate diameter by method A and to overestimate by method B.

The distribution of the 100 fibers based on estimates of diameters by method A agrees closely with that based on actual measurements, whereas that obtained by method B is distinctly different.

An analysis of variance shows that 82.3 percent of the variance in the scores obtained by method A is due to differences among wool samples, whereas in method B only 70.7 percent is due to this source of variance.

Intraclass correlations between scores of samples on different days, between scores of the same judge on different days, and between scores of the same sample by different judges are all highly significant for both methods. In all cases the correlations for method A are higher but the differences are small. A study of the placements by individual judges indicates that all judges were working with about the same degree of accuracy within each method.

The findings indicate that the use of method A results in estimates of fiber diameters that are nearer the actual measurements, and that this method is more desirable than method B in all types of work in which the detection of small differences in fiber size is essential.

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CYTOLOGY OF PARTHENOGENESIS IN *POA PRATENSIS*¹

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INTRODUCTION

Since Müntzing (8)³ announced in 1932 that seed is set without gametic union (apomixis) in certain Swedish biotypes of *Poa pratensis* L. and *P. alpina* L., and with gametic union (amphimixis) in certain Swiss biotypes of *P. alpina*, several other investigators have reported evidence that apomixis occurs in *P. pratensis*. The cytological studies heretofore published, however, do not adequately explain apomixis in these species. The observations herein described pertain to the cytology of apomictic seed development in *P. pratensis*.

REVIEW OF LITERATURE

The studies of Müntzing (8) on Swedish biotypes of *Poa pratensis* showed that plants of different biotypes (or progeny from different plants) might have different somatic chromosome numbers, whereas plants of the same biotype (or the progeny from one plant, in one known instance) possess the same maternal chromosome number. He found also that plants with a common maternal origin were extremely uniform in morphological type and that the chromosome behavior in the meiotic divisions of the microsporocytes was frequently irregular although this irregularity had no apparent effect on fertility. Müntzing (9), in a study of twin seedlings, found that the two plants obtained from one seed usually had approximately the same number of chromosomes but that it was not uncommon for one member of such a pair to be approximately triploid in respect to the diploid chromosome number of its twin.

Nilsson (10, 11, 12) found that self-fertility might vary from 0 to 78.9 percent in different plants of *Poa pratensis* and that the fertility of open-pollinated plants might vary from 2.6 to 78.1 percent. He suggested that fertility in individual plants is affected by the environment and that in some plants sterility is due to defective sex organs. He concluded that the seeds of *P. pratensis* are set apomictically in nearly all cases. This species, however, presents a case of pseudogamy since pollination must be effected to induce seed development. Occasional intermediate forms appear, which Nilsson suggested result from gametic union.

¹ Received for publication July 1, 1939. The investigation herein described is a part of a project on the improvement of pasture grasses that is being conducted cooperatively between the Department of Agronomy, Wisconsin Agricultural Experiment Station, and the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

² The author lost his life in the sinking of the S. S. *Athenia* on September 3 or 4, 1939, while on his return from the Seventh International Congress of Genetics, which met at Edinburgh, Scotland, August 23-29, 1939. In submitting the manuscript of the present paper the author asked that acknowledgment be made of the assistance of Arnold Lueck and Paul Ozanne, who aided in making the cytological preparations; of Prof. O. S. Aamodt, who contributed in many ways toward the completion of the work; and of Prof. C. E. Allen, who aided in the final preparation of the manuscript.

³ Italic numbers in parentheses refer to Literature Cited, p. 360.

Åkerberg (3) also concluded that apomixis commonly occurs in *Poa pratensis*, although he has found four biotypes that regularly reproduce sexually. He found pollination necessary to initiate apomictic seed development; the pollen of *P. alpina* was nearly as effective in providing the stimulus to the development of the ovule of *P. pratensis* as was its own pollen. Fertilization is thought to occur occasionally in apomictic plants, since intermediate types appear. It is suggested from the chromosome number of one hybrid, that it arose from a fusion of an unreduced egg and a reduced male gamete (1). Another hybrid that Åkerberg obtained from pollinating unemasculated florets of *P. pratensis* with pollen from *P. alpina* suggested by its chromosome number that it arose from fertilization of a reduced egg by a reduced male gamete (2).

Cytological studies on the embryo-sac development have been made by Andersen (4) and Armstrong (5). Since Andersen's study was made prior to the suggestion that apomixis occurs in *Poa*, she naturally did not consider seed development from this point of view. She stated, however, that "fertilization was not observed by the author, although the slides were carefully examined for this detail."

Armstrong (5) found that plants from different strains of Canadian and European origin might have different chromosome numbers. All the strains that he studied gave evidence of the occurrence of meiosis in macrospore mother cells. He concluded, from the presence of paired chromosomes at diakinesis in macrospore mother cells and from the good germination of pollen, that fertilization occurs in two strains, Mammoth from Ontario and No. 994 from Aberystwyth. It is interesting to note that he found the plants of the Mammoth strain morphologically uniform.

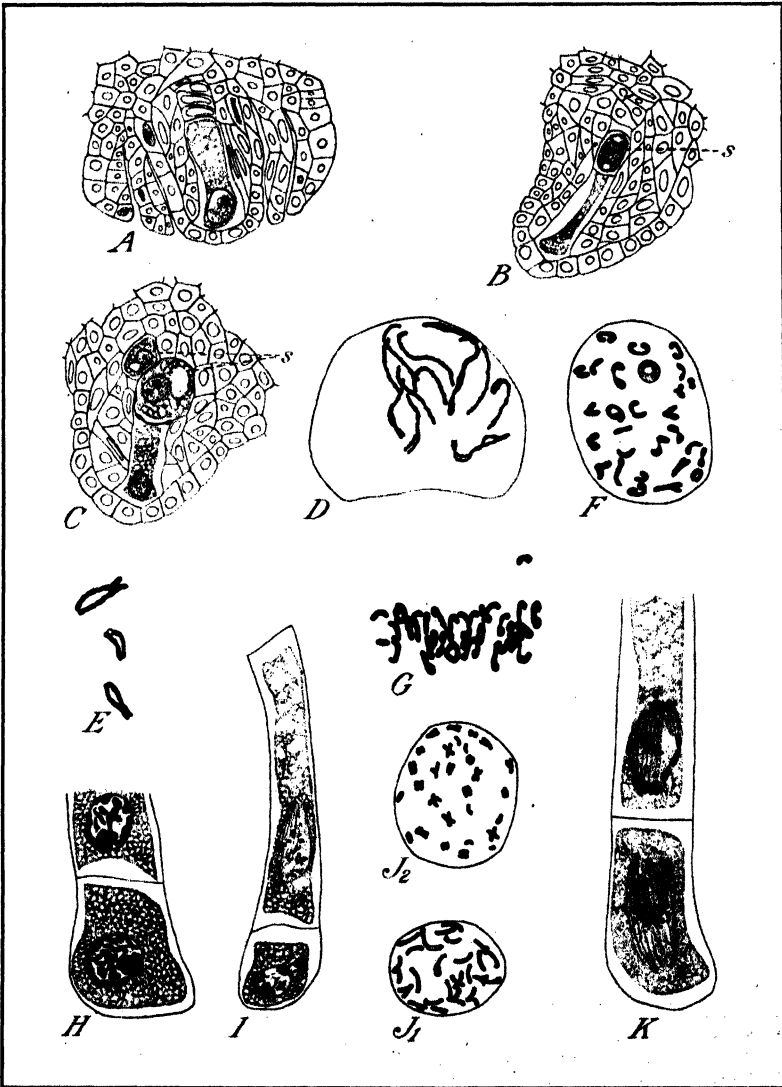
Other reported apomictic species in the Gramineae are: *Poa palustris* L. (*P. serotina* Ehrh.) (7); *P. alpigena* (E. Fries) Lindm., *P. glauca* Vahl., *P. arctica* R. Br. (6); *Calamagrostis obtusata* Trin. (15); and *Nardus stricta* L. (14). The complete cytological facts are not known for any of these species.

MATERIALS AND METHODS

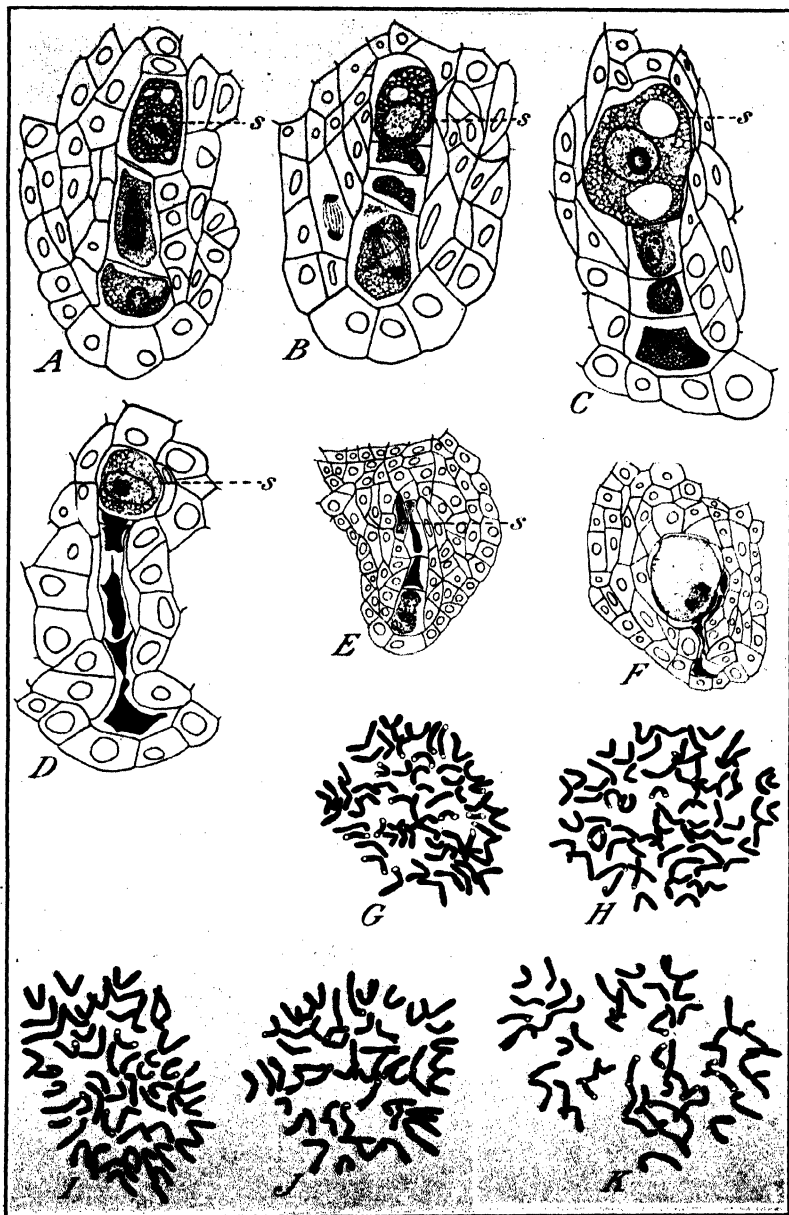
Seed of *Poa pratensis* collected from some of the oldest permanent pastures in Wisconsin was grown as single clones in the agronomy greenhouses and on the university farm at Madison, Wis. Most of the material for cytological study was collected from plants growing in the greenhouses during the past three winters, although some material was studied from collections made during summers in the field nursery.

In order to avoid confusion due to collecting material from plants differing among themselves in their method of reproduction, as many stages as possible were studied in material collected from the same clone. Altogether 5 clones of different biotypes have been studied in detail. In addition, material from 1 clone from each of 12 different biotypes was collected in the field nursery for a study of embryo development in the embryo-sac just before pollination.

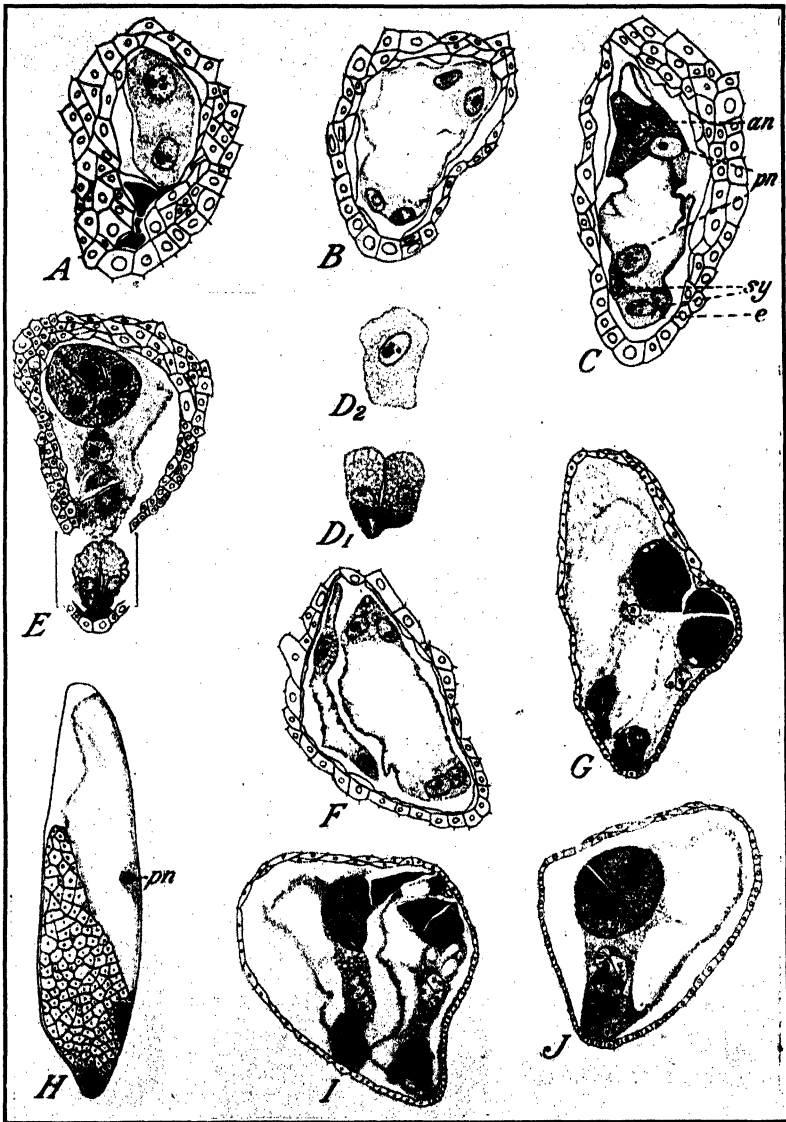
Individual florets were separated from the spikelets with the aid of a dissecting microscope, submerged for not more than 1 second in a solution of Carnoy's solution (6 parts absolute alcohol : 1 part glacial acetic acid : 3 parts chloroform), and then placed directly into Munt-



A, Young ovule with developing integuments and macrospore mother cell. $\times 218$. *B*, Portion of nucellus, showing macrospore mother cell and differentiated somatic cell (*s*). $\times 218$. *C*, Same as *B*, but showing macrospore mother cell and two differentiated somatic cells (*s*). $\times 218$. *D*, Section through nucleus of macrospore mother cell illustrated in *A*, showing paired chromosome at diplotene. $\times 1,640$. *E*, Portion of nucleus of a macrospore mother cell in late diakinesis. $\times 1,640$. *F*, Nucleus of macrospore mother cell (taken from plant shown in plate 2, *I* and *J*). $\times 1,640$. *G*, Metaphase of first meiotic divisions in macrospore mother cell. $\times 1,640$. *H*, Portion of daughter cells formed by first meiotic division; nuclei in interkinesis. $\times 629$. *I*, Daughter cells; micropylar nucleus in interkinesis; chalazal nucleus in anaphase. $\times 462$. *J*₁ and *J*₂, Second meiotic division; micropylar nucleus in early prophase, showing approximately 25 chromosomes (*J*₁); chalazal nucleus in late prophase, showing approximately 20 chromosomes (*J*₂). $\times 1,640$. *K*, Portion of daughter cells, second meiotic division; micropylar nucleus in anaphase. chalazal nucleus in telophase. $\times 629$. Drawings made with camera lucida at table level. All figures arranged with micropylar end of embryo sac toward bottom of page.



A. Portion of nucellus, showing two cells formed as result of first meiotic division; nucleus of micropylar cell in interkinesis and other in metaphase of second division; differentiated somatic cell (*s*). $\times 342$. **B.** Same as **A.** but showing three cells formed as result of meiotic divisions (micropylar cell failed to complete second division); differentiated somatic cell (*s*). $\times 342$. **C.** Same as **B.** but showing further enlargement of somatic cell (*s*). $\times 342$. **D.** Same as **B.** but showing four disintegrated macrospores and differentiated somatic cell (*s*) separated from macrospores by vegetative cell. $\times 342$. **E.** Same as **B.** but showing three cells in process of disintegration (micropylar cell failed to complete second division); early differentiation of somatic cell (*s*). $\times 180$. **F.** Same as **B.**; first division of nucleus in embryo sac and disintegrated macrospores. $\times 180$. **G.** Metaphase; first division of nucleus in embryo sac, showing approximately 63 chromosomes. $\times 1,112$. **H.** Metaphase; root tip of same plant as **G.**, showing approximately 79 chromosomes. $\times 1,112$. **I.** Same as **G.** from another biotype, showing approximately 53 chromosomes. $\times 1,112$. **J.** Metaphase; root tip from same plant as **I.**, showing approximately 53 chromosomes. $\times 1,112$. **K.** Metaphase; root tip from same plant as in plate 1, **J**, and **J**, showing approximately 54 chromosomes. $\times 1,112$.



A, Portion of nucellus, showing two-nucleate embryo sac and three disintegrated macrospores. $\times 199$. B, Same as A, showing four-nucleate embryo sac. $\times 199$. C, Same as A, showing complete embryo sac, antipodals (an), polar nuclei (pn), synergids (sy), and egg (e). $\times 199$. D₁, Synergids, and D₂, egg, from same embryo sac. $\times 199$. E, Portion of nucellus, showing embryo sac with two-cell proembryo; two synergids, displaced by amount indicated to permit of their being drawn. $\times 141$. F, Portion of nucellus with two developing embryo sacs: one binucleate, the other four-nucleate. $\times 199$. G, Composite drawing from two sections, showing two complete embryo sacs each containing a proembryo. $\times 97$. H, Composite drawing of later stage in seed development, showing two proembryos surrounded by endosperm of one embryo sac; two polar nuclei (pn) of second sac are unfused. $\times 26\frac{1}{2}$. I, Composite drawing showing portion of nucellus and two embryo sacs, one of which possesses two polar nuclei; the other, five. $\times 97$. J, Portion of nucellus with single embryo sac, showing four polar nuclei. $\times 97$.



A, Photomicrograph of section of ovule collected just before pollination, showing proembryo with several cells. $\times 231$. *B*, Photomicrograph of section of ovule collected after anthesis, showing two embryos. $\times 150$.

zing's (8) modification of Navashin's solution or another modification composed of equal parts of two solutions, one of which consisted of 90 cc. of water, 10 cc. of glacial acetic acid, and $1\frac{1}{2}$ gm. of chromic acid, and the other, of 40 cc. of formalin and 60 cc. of water. The air was removed with an aspirator. Fixation may be further enhanced by completely removing all floral parts and quickly submerging the pistils in the Carnoy fluid before placing them in the final fixing solution. This latter procedure was found especially useful when it was necessary to cut the ovules at particular angles in order to obtain polar views of the reduction divisions or of the first nuclear division in the embryo-sac mother cell. Root tips were fixed in Muntzing's solution. All material was left in the fixing fluid for from 18 to 24 hours and then washed in three or four changes of 70-percent alcohol (13). It was dehydrated in the usual manner, cleared in cedarwood oil, and embedded in paraffin. Root tips were cut at 10μ and stained in crystal violet iodine. Florets and pistils were cut from 10μ to 20μ , stained in Heidenhain's iron-alum haematoxylin, and destained in a saturated aqueous solution of picric acid.

OBSERVATIONS

The ovule in the basal florets of the spikelets, at the time the panicle begins to emerge from the sheath, consists of an outer and inner integument, each composed of two layers of cells, and a nucellus with a well-differentiated macrospore mother cell (pl. 1, *A*). A median section of an ovule at this stage invariably shows a single elongated, very conspicuous macrospore mother cell with the nucleus located usually near the micropylar end, or in some instances near the chalazal end. The cytoplasm is usually denser at the micropylar end than at the chalazal end of the cell.

In some instances at this early stage, one cell (pl. 1, *B*), or less frequently two cells (*C*), of the nucellus, near the chalazal end of the macrospore mother cell, differ conspicuously from the surrounding cells of the nucellus. The differentiated nucellar cell (or cells) is spherical, stains more darkly and, except for one or two characteristic vacuoles, contains denser cytoplasm than the surrounding cells of the nucellus. The significance of this cell will be discussed later.

The nucleus of the macrospore mother cell undergoes, so far as can be determined, a typical first meiotic division. Some tangential sections (pl. 1, *D*) clearly show paired chromosomes at diplotene. At later stages (*E*) the paired chromosomes appear to be held together by chiasmata. Stages in diakinesis (*F*) show that the chromosomes in most instances are associated in pairs, although univalents are frequently present. In one metaphase figure of the first reduction division (*G*) most of the chromosomes are associated in pairs, but there are three trivalent chains so arranged on the spindle that the two end chromosomes of the chain in each case will pass to the same pole; one chain of either three or four chromosomes is so arranged that the end chromosomes will pass to opposite poles; and three or four univalents are present. The first meiotic division forms two cells of which the one nearer the micropyle is usually the smaller (*I*). The nucleus of each daughter cell passes into interkinesis (*H*).

During the second meiotic division the chalazal cell precedes the micropylar cell in division. In one instance (pl. 1, *I*) the nucleus in the micropylar cell is in interkinesis when the one in the chalazal cell

is in anaphase; in another (J_1), the micropylar nucleus is in an early prophase, and the chalazal nucleus (J_2) is in a late prophase. J_1 and J_2 show that the number of chromosomes in each daughter nucleus is approximately one-half the somatic number of approximately 54 in the root tip of the same plant (pl. 2, K). In another instance (pl. 1, K), the nucleus of the micropylar cell is in anaphase and the nucleus of the chalazal cell in telophase. Univalents are frequently observed either passing precociously to the poles or remaining at the equator. In either case they frequently fail to be included in a daughter nucleus. Either daughter nucleus, then, may not receive a complete chromosome complement.

The second meiotic division in the chalazal cell regularly forms two haploid macrospores, both of which soon disintegrate. In most of the observed cases this second division is not completed in the micropylar cell because of the precocious disintegration of the cell (pl. 2, B, C, E). Consequently, only three cells are formed from the macrospore mother cell, although in one instance (D) four macrospores were observed. In all observed instances all the macrospores thus formed from the macrospore mother cell disintegrate and do not function in the development of the embryo sac.

A cell of the nucellus near the chalazal end of the macrospore mother cell has been described (p. 353) as being conspicuously different from the surrounding cells. In some instances (pl. 1, B, C) one cell or occasionally two cells are differentiated as early as the beginning of meiosis in the macrospore mother cell. Not infrequently, differentiation is not conspicuous until the meiotic divisions are completed (pl. 2, E). Plate 2, A , shows that of the two cells toward the micropyle, the nucleus of one is in interkinesis and the nucleus of the other is in metaphase, while the third cell, largest and inward, has been separately differentiated. In plate 2, B and C , the three micropylar cells that were derived from the macrospore mother cell are beginning to disintegrate. In the one nearest the micropyle, nuclear division has just been completed. The fourth and innermost cell has been differentiated separately. In another instance (pl. 2, D), four macrospores had been formed by two completed divisions and had completely collapsed. At the chalazal end of the macrospore row is a separately differentiated cell, separated from the row of disintegrated macrospores by a vegetative nucellar cell.

The differentiated nucellar cell just described is destined to function as the initial cell of the embryo sac (pl. 2, F). This conclusion is confirmed by the chromosome counts during the division of the primary nucleus of the embryo sac. In one instance (G) approximately 63 chromosomes were observed in this division. The chromosome number in cells of a root tip (H) of the same plant is approximately 70. In consequence of the high number of chromosomes and the resultant difficulty in distinguishing them, it is probable that the remainder of the diploid complement in the nucleus of the embryo sac was hidden from view. In another case, approximately 53 chromosomes were observed in nuclear division both in the initial cell of the embryo sac (I) and in a cell of a root tip (J) of the same plant. In several other cases a sufficiently accurate count of the chromosomes in the initial cell of the sac could be made to demonstrate the presence of the diploid number.

A two-nucleate embryo sac and three disintegrated macrospores are shown in plate 3, *A*, a four-nucleate sac in plate 3, *B*, and a completed eight-nucleate seven-cell sac in plate 3, *C*.

The mature embryo sac consists of three large, darkly staining antipodal cells at the chalazal end, a single central cell containing two polar nuclei, and the egg apparatus (egg and two synergids) at the micropylar end. The nuclei of the antipodal cells may divide and if cell division does not occur the cell in question remains binucleate. In some cases cell division follows to form ultimately five or six antipodal cells.

The synergids (pl. 3, *D*₁ and *E*) enlarge and become pear-shaped, with the apex of each projecting toward the micropyle. They lie side by side directly beside the egg. The cytoplasm in the basal portion of each synergid becomes very fibrous in appearance, constituting the filiform apparatus often described for synergids. A nucleus lies near the center of each synergid, and as the two cells disintegrate the nuclei maintain their structure longer than does the cytoplasm. In some instances it appears that the two synergids in the process of disintegration fuse into one darkly staining body.

The cytoplasm of the egg (pl. 3, *D*₂) becomes coarsely vacuolated; the nucleus lies near the center of the cell surrounded by denser cytoplasm. At the apical end the cytoplasm assumes regularly a more coarsely alveolar appearance, which seems to disappear when the cell divides.

The egg divides to form a proembryo in many of the first, second, or third florets of the spikelet before anthesis begins (pl. 3, *E*). The two synergids shown in *E* have been displaced by the amount indicated to permit of their being drawn. The proembryo shown in the photomicrograph (pl. 4, *A*) is larger than the one in the drawing (pl. 3, *E*), but the florets from which these figures were obtained were at approximately the same stage of flowering when fixed. In this instance the synergids have begun to disintegrate and have lost their identity. These observations have been made repeatedly in material fixed in the greenhouse under conditions that excluded the possibility of the presence of pollen in the air, and in material fixed in the field 1 or 2 days before the floral parts had opened to permit the exposure of the stigma. There seems no doubt that pollen is unnecessary to initiate development of the egg into an embryo. Since it has been shown that the nuclei of the embryo sac, including the egg nucleus, are all diploid, it is apparent that the functioning of the egg is not dependent on gametic union. The external stimulus to the egg, if one is necessary, is apparently exerted from within the embryo sac.

The two polar nuclei (pls. 3, *H*; 4, *A*) lie together in the dense cytoplasm of the embryo sac midway between the antipodals and the proembryo. Fusion of the polar nuclei is not completed until the proembryo consists of several cells, and occurs invariably after anthesis. Fusion is accomplished by dissolution of the adjacent membranes to form the primary endosperm nucleus, which later divides to form the endosperm nuclei. The cells of the endosperm, thus formed, would possess a $4n$ number of chromosomes in contrast to the $3n$ number usually found in sexually reproducing species.

It is not certain whether pollination is associated with endosperm development. Pistils examined a few hours after pollination show pollen grains germinating freely on the stigma and pollen tubes

growing down the style. In no case, however, have pollen tubes been observed in tissue of the ovary, or in the embryo sac. Florets that were emasculated and not pollinated have in a few instances developed seeds, but it is possible that in these instances the stigma was accidentally pollinated during the act of emasculation. Florets that were open-pollinated in the greenhouse occasionally contained aborted ovules, and examination has shown proembryos but no endosperm. The two unfused polar endosperm nuclei were still frequently visible in such cases. The failure of the polar nuclei to fuse and to bring about the development of an endosperm may have been due to a failure of pollen tubes to stimulate endosperm development.

POLYEMBRYONY AND ATYPICAL EMBRYO SACS

Sections of young ovules not infrequently show two embryo sacs in course of development (pl. 3, *F*). The initial cells from which such sacs develop have not been observed in division, but it seems very probable from observations of ovules containing single embryo sacs that two sacs may arise independently from two differentiated nucellar cells, such as are sometimes present (pl. 1, *C*). Consequently, the nuclei in both embryo sacs would be expected to possess identical diploid chromosome complements. Andersen (4) concluded that polyembryony results from the functioning of two embryo sacs and that each embryo sac develops either from two macrospores in the same row or from two macrospores derived from separate macrospore mother cells.

One embryo sac, presumably that favored by position, may grow faster than its twin; the larger one develops in the usual manner, while the smaller develops irregularly and consequently may not produce a proembryo. If the development is not too irregular, each will produce a proembryo (pl. 3, *G*). In all observed instances in which two embryos were present there was evidence of the previous presence of two embryo sacs; for example, two sets of antipodals, or two pairs of polar nuclei, or both. The ovule illustrated in plate 3, *H*, contains two proembryos and a mass of endosperm tissue formed by division of the primary endosperm cell of one embryo sac. The previous presence of a second sac is evidenced by the two polar nuclei incompletely fused.

The embryo nearer the micropyle may be large and typical in shape; its twin embryo smaller, atypical, and located in an unusual position (pl. 4, *B*). Such instances, however, do not necessarily imply a difference in origin of the smaller embryo. These instances may be explained by the more rapid growth of one embryo and of its endosperm, in consequence of which the smaller embryo, in a less favorable position for growth, is crowded against the wall of the nucellar cavity.

There remains the possibility that one of the twins may have developed parthenogenetically from a haploid egg, in which case it would be expected to be weaker than the diploid twin. Müntzing (9) found one haploid plant which he suggested arose as a twin from the parthenogenetic development of a haploid cell. He found also in some instances that the two members of a pair, in the seedling stage, differ in size and vigor, perhaps in consequence of the more favorable position of the larger. He found the chromosome number of twin seedlings to be usually the same, but occasionally one was approximately triploid

with respect to its diploid twin; and if there was a difference in size, the triploid seedling, more frequently than the diploid, was the weaker of the two. Müntzing suggested that the triploid arose from the fertilization of a diploid egg.

The egg in each embryo sac (in case two sacs are present) frequently develops into a proembryo before pollination, and under such circumstances fertilization of the egg in either embryo sac obviously could not occur. The chromosome number would be the same in the two embryos (omitting the possibility of the development of a haploid embryo sac from a macrospore). If one or both eggs did not begin to develop until sufficient time had elapsed for the pollen tubes to reach the embryo sacs, one or both eggs might be fertilized. Müntzing (9) found both members of one set of twin seedlings to be approximately triploid. It seems reasonable to assume that the eggs in the weaker embryo sac might delay parthenogenetic development longer than those in the more vigorous one, and consequently provide opportunity for gametic union. Under such circumstances triploidy will be more frequently associated with the smaller of twin seedlings than with the larger.

There is no evidence that embryos originate by sporophytic budding from the nucellus.

Embryo sacs, when occurring either singly or doubly, occasionally contain more than the eight nuclei. In some instances an apparently functional embryo sac, in addition to the antipodals, two-nucleate primary endosperm cell, and egg apparatus, consists of single cells or nuclei lying in the cytoplasm of the nuclear cavity. It is probable that the condition might become too atypical for an embryo sac to function.

More than two polar nuclei are occasionally seen in process of fusion. In one instance (pl. 3, *J*) there were four polar nuclei; and in another (pl. 3, *I*), there were five polar nuclei in one embryo sac and two in a companion sac. The origin of these additional nuclei or cells is not known, but it seems probable that they result from nuclear divisions during embryo-sac development. It seems improbable that any of the nuclei shown fusing with the two polar nuclei came from a pollen tube.

DISCUSSION

The evidence presented by Müntzing (8), Nilsson (12), and Åkerberg (3) indicates that gametic union does not regularly occur in Swedish biotypes of *Poa pratensis*. According to Åkerberg and Nilsson, however, pollination is necessary to initiate seed development, and in a few cases they found evidence that pollination was followed by a union of male and female gametes. The chromosome number of one hybrid plant suggests that it arose from the union of an unreduced egg and a reduced male gamete (1). Müntzing (9) found that occasionally one member of a twin pair was approximately triploid and suggested that in such a case a diploid egg was fertilized by a haploid male gamete. In two instances the egg was thought to have been haploid. In one of these instances the egg was presumably fertilized by a haploid male gamete of *P. alpina* (2), while in the other the haploid egg presumably developed parthenogenetically (9). Åkerberg (3) found four biotypes that appeared to reproduce regularly by gametic union.

The cytological observations on *Poa pratensis* herein described show that in the biotypes studied the single macrospore mother cell underwent meiosis in the usual manner and formed haploid macrospores (usually three, since the second meiotic division was often incomplete). In all the observed instances the three or four macrospores so formed disintegrated. Andersen (4) reported, without presenting evidence, that the macrospore mother cell formed a row of four macrospores and that usually the chalazal macrospore formed the embryo sac although in several observed ovules the micropylar macrospore functioned. Armstrong (5) observed meiosis and concluded that one of the macrospores thus formed develops a haploid embryo sac and that meiosis ipso facto implies sexual reproduction.

The initial cell of the embryo sac is differentiated from among the vegetative cells of the nucellus while the meiotic divisions are under way, and develops into a typical eight-nucleate, seven-cell embryo sac. Consequently the nuclei of the embryo sac, including the egg nucleus, possess the same diploid chromosome complement as do the nuclei of the maternal parent. The egg is capable, consequently, of developing into an embryo without restoration of the diploid chromosome number by fertilization. Under these circumstances all the plants grown from seeds collected from a single plant possess the same diploid complement of chromosomes, and obviously are, therefore, similar in morphological type. Müntzing (8) has found that 10 plants examined in a progeny from a single plant all had the same maternal chromosome number. Observations made by the author indicate that all the plants (with few possible exceptions) grown from seeds collected from a single plant are alike in growth form and habit. Since the embryo sac develops from a vegetative cell of the nucellus without meiosis, its origin may be considered an instance of apospory. The embryo develops from the egg by parthenogenesis.

Cytological observations have not confirmed the conclusions of Åkerberg (1) and Nilsson (12) that pollination is necessary to induce seed development. These investigators based their conclusions on the lack of seed setting in emasculated florets, and obviously were not able to separate the two phases of the development of a seed, i. e., embryo development and endosperm development. The embryo of *Poa pratensis* develops in many instances before pollination, but since endosperm development does not begin until after pollination the effect of pollen or pollen tubes on endosperm development is not easy to determine. A comparatively few florets have been emasculated by the author, and some of these have produced seeds. In the emasculation the floret may have been injured or the pistil unintentionally pollinated. According to Åkerberg (1) and Nilsson (12), the environment also may affect seed development. Consequently, negative results might be due to any of several causes. There is some evidence to indicate that seed abortion is due to the lack of endosperm development, and it may be that in such instances pollen failed to germinate on the stigma.

The egg frequently begins development before pollination is effected, but occasionally development does not begin until after pollination. Since pollen tubes have been observed in the style, although not in the embryo sac, it is possible that occasionally one reached the embryo

sac before parthenogenetic development began and that a diploid egg was then fertilized. Circumstances such as this may lead to the production of an occasional hybrid as reported by Åkerberg (1), and of triploid twin plants such as were found by Müntzing (9). Chromosome disjunction during meiosis in the microsporocytes is extremely irregular, and in many instances chromosomes are lost from the daughter nuclei. It is not known whether chromosome-deficient pollen grains germinate on the stigma, although a deficiency appears to have no effect upon the development of pollen grains. If a gamete possessing a deviating chromosome number effects fertilization, the resulting zygote will be only approximately triploid. Müntzing (9) is not certain of the exact chromosome number in twin plants of *Poa pratensis*, but he suggests that the number is only approximately triploid. Once the hybrid is produced, the newly combined chromosome complements, either euploid or aneuploid, may be transmitted to future generations by parthenogenesis. Diploid parthenogenesis and occasional fertilization of the egg by a sperm possessing a varied chromosome number can produce the singular characteristics of *P. pratensis*: (1) Extreme polymorphism and varying euploid and aneuploid chromosome numbers of different biotypes and (2) constant morphological type and chromosome number in plants of the same biotype.

SUMMARY

Median sections through the micropylar region of young ovules of *Poa pratensis* invariably show a single, elongated, very conspicuous macrospore mother cell. The nucleus of this cell undergoes meiosis, and haploid macrospores are formed—usually three, since frequently the micropylar cell does not complete the second meiotic division. In observed instances, all the macrospores subsequently disintegrate.

The embryo sac develops, without meiosis, from a cell of the nucellus, which is located near the chalazal end of the macrospore mother cell. The typical mature embryo sac consists of three antipodal cells, a primary endosperm cell containing two nucelli, and the egg apparatus (egg and two synergids).

The diploid egg develops into a proembryo by parthenogenesis; the development begins frequently before pollination. (Possible exceptions are noted in the text.) Since endosperm development was not observed to begin until after pollination, it may be that pollination or the growth of pollen tubes in stylar tissue is necessary for endosperm development and consequently for seed development. Pollen tubes have not been observed in the embryo sac.

Diploid parthenogenesis and occasional fertilization of the egg by a sperm possessing a varied chromosome number can produce the singular characteristics of *Poa pratensis*: (1) Extreme polymorphism and varying euploid and aneuploid chromosome numbers between different biotypes and (2) constant morphological type and chromosome number in plants of the same biotype.

Two embryos are occasionally produced by the functioning of two embryo sacs. Each embryo sac appears to develop independently from separate somatic cells of the nucellus. There is no evidence that embryos arise by sporophytic budding from the nucellus.

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JOURNAL OF AGRICULTURAL RESEARCH

VOL. 60

WASHINGTON, D. C., MARCH 15, 1940

No. 6

RELATION OF FOOD TRANSLOCATION TO MOVEMENT OF VIRUS OF TOBACCO MOSAIC¹

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INTRODUCTION

Previous studies (2)² have shown that when raspberry plants are infected by the virus of leaf curl or when sugar beet plants are infected by the virus of curly top, virus movement takes place in the phloem of the invaded plants. There is also strong evidence that these two viruses are closely limited to the phloem of their respective host plants and that their movement through the plants is correlated with the translocation of organic food materials. The weight of evidence indicates further that the phloem network of the plant is the avenue through which rapid invasion is accomplished by all viruses that are able to produce systemic infection. Whether the correlation between virus movement and food translocation shown with the two viruses already mentioned exists with other viruses, especially with viruses such as that of tobacco mosaic, which is not limited to the phloem, has not been determined definitely. Conflicting conclusions have been reached from the evidence that has been obtained thus far.

Holmes (10) presented evidence that seems clearly to indicate a relationship between the translocation of carbohydrates and the movement of the virus of ordinary tobacco mosaic in tobacco. Samuel (17) found an apparent correlation between food translocation and movement of the virus of tobacco mosaic in tomato. Caldwell (5, 6), however, working with the viruses of aucuba and tobacco mosaic in tomato and tobacco, found no correlation between virus movement and food transport. He reached the conclusion that the viruses moved independently of food materials and that under certain conditions virus movement was apparently in directions opposite to that of the metabolites. Grainger (9) reached conclusions similar to those of Caldwell and suggested that in his experiments the virus of tobacco mosaic moved equally in all directions in which channels rendered it free to move. He considered that the virus spread through the plant from the point of introduction at a logarithmic rate.

Conflicting conclusions regarding the movement of different viruses led Raber in his review of a book by Curtis (8) to suggest that there is no uniformity in the way in which viruses move through the plant, each virus perhaps exhibiting a specific behavior in this respect.

Invasion of plants by viruses is of general interest in the field of plant pathology, and because of the potential significance of plant viruses as indicators of food translocation, virus movement is of

¹ Received for publication June 20, 1939.

² Italic numbers in parentheses refer to Literature Cited, p. 389.

particular interest in relation to the general subject of translocation of organic materials in plants. This paper reports the results of experiments on the movement of the virus of tobacco mosaic (tobacco virus 1, Johnson)³ in *Nicotiana tabacum* L. and *N. glauca* Graham under conditions similar to those under which the movement of the virus of curly top of sugar beet was studied. (4).

ACROPETAL AND BASIPETAL MOVEMENT IN STEMS

The early work of Allard (1) showed that the passage of tobacco mosaic virus from the point of inoculation near the leaf apex of young tobacco plants to the stem required at least 3 days. Most of the published information on the movement of the virus of tobacco mosaic has been derived from the study of movement in tobacco and tomato plants growing in a normal upright position under more or less normal conditions for photosynthetic activity and food transport. In experiments with tobacco, Holmes (10) showed that when the virus was introduced into a leaf its movement was very slow until it entered a vein, after which movement was more rapid and was chiefly in the direction of the stem. After the virus passed out of the inoculated leaf the entire stem was invaded in a relatively short time.

Caldwell (5) found that when the virus of tobacco mosaic passed out of an inoculated leaf of a tobacco plant it moved first in the direction of the top. However, Samuel (17) found that when the virus moved into the stem of a tobacco or tomato plant it usually moved first in the direction of the root.

Results of numerous tests conducted during the course of the work reported in this paper show that when the virus of tobacco mosaic was introduced into a leaf at the top of a Turkish tobacco plant it moved into the root system within a few days. When the virus was introduced into a leaf near the middle of a stem of a relatively large plant, it moved into the top and the root portions within a period which usually did not exceed 4 to 8 days.

In view of the evidence available, it seems certain that under normal conditions both acropetal and basipetal movements of virus occur in the stems of tobacco and tomato plants within a relatively short time after virus introduction. It should be emphasized, however, that it has not been proved that movements in the two directions occur simultaneously.

It seems probable that when virus enters a stem the direction of its movement may depend upon factors operating in the stem at the time of entry and hence movement may be in either direction, depending on internal conditions in the plant at the time of entry. If virus movement is correlated with food transport, the directional movements that have been observed in the stems of tobacco may readily be explained by assuming that in the normal plant there is a diurnal directional reversal of flow of organic materials in these plants. The virus then would move either acropetally or basipetally, depending on the direction of food flow at the time the virus entered the stem, but it might be carried into the two extremes of the plant in the course of 24 hours.

³ The virus used in these experiments was obtained from Dr. William N. Takahashi, University of California, who had previously obtained it from Dr. James Johnson, University of Wisconsin.

In plants in which frequent reversals of food flow and virus movement occur, a determination of a possible correlation between movement of the two types of materials would be difficult. However, if a plant could be manipulated in such a way as to insure continuous unidirectional flow of food materials in one of its parts a study of the movement of virus in such a plant would be of interest. With this object in view, the following experiments were made.

EXPERIMENTS WITH *NICOTIANA TABACUM*

Potted plants of *Nicotiana tabacum*, Turkish variety, about 24 inches tall, were bent so that their stems were in a horizontal position. This resulted in the production of a number of suckers at the base of each stem. One sucker near the base of each plant was retained and the others were removed when small. By this method it was hoped to produce a plant with two growing points, in which the basal shoot, being in an upright position and more advantageously located for growth, would tend to drain the root system of food reserves and prevent outward movement of food materials in the horizontal main stem. Growth of the basal suckers was more rapid in most plants than that of the horizontal stems, suggesting that the basal shoots were deriving a part of their carbohydrate supply from the rest of the plant, at least in the earlier stages of their development.

When the basal suckers were about 2 inches tall the plants were divided into two lots. The plants of the first lot were inoculated on the small leaves at the top of the main stems and the plants of the second lot were inoculated on the basal suckers. The plants were allowed to develop without further treatment, and the time of appearance of symptoms on the two growing points of each plant was noted.

The movement of the virus in a basipetal direction in the main stem was relatively fast, as shown in the results presented in table 1. The entire length of the stem (more than 24 inches) was traversed, and symptoms were produced on the basal sucker in an average period of 6.8 days. This period was only 2 days longer than that required for the production of symptoms on the basal shoots inoculated directly. It may be assumed, therefore, that in this experiment the virus moved basipetally at a rate of approximately 24 inches in 48 hours.

TABLE 1.—*Acropetal and basipetal movement of virus in Nicotiana tabacum and N. glauca*

Species of <i>Nicotiana</i>	Plants tested	Place of inoculation	Direction of movement	Average period for appearance of symptoms on—	
				Basal sucker or graft	Growing points at top of plants
	Number			Days	Days
<i>N. tabacum</i>	20	Top.....	Toward root.....	6.8	6.3
	20	Base.....	Toward top.....	4.8	35.5
<i>N. glauca</i>	10	Top.....	Toward root.....	8.4	5.2
	10	Base.....	Toward top.....	5.4	197.5

¹ 7 of the 10 plants included in this average showed no symptoms on the top graft when the experiment was discontinued 224 to 252 days after the plants were inoculated.

Movement from the inoculated basal suckers acropetally in the main stem was relatively very slow. An average period of 35.5 days was required for the appearance of symptoms in any of the parts of these shoots. During this period the plants had blossomed and produced seed pods that were well advanced toward maturity at the time symptoms were evident.

EXPERIMENTS WITH *NICOTIANA GLAUCA*

Plants of *Nicotiana glauca* have generally been considered to be able to harbor the virus of tobacco mosaic without manifesting distinct mottling. None of the plants used in these experiments has shown mottling that appeared to be due to the presence of the virus of tobacco mosaic. Since symptoms cannot be relied upon to indicate infection, all experiments on the movement of the virus in this species were conducted with plants in which grafts of Turkish tobacco were placed at the points of inoculation and at the points where it was desired to detect the presence of the virus through expression of symptoms.

Large plants with a single stem were selected for the tests. The stems were cut to a height of 3 feet. Three-inch pieces of stem from healthy Turkish tobacco plants were grafted into the top and base of each plant. The stems were then bent sufficiently to prevent contact between the shoots subsequently produced from the two grafts. When the new shoots from the grafts were 3 to 6 inches long the plants were divided into 2 lots of 10 plants each. Virus was introduced into the basal grafts of 1 lot and into the top grafts of the other. A record of the time of appearance of symptoms on the grafts of each plant was kept.

Table 1 also shows that the basipetal movement from inoculated grafts through the stem of *Nicotiana glauca* was comparable to that in previous experiments with Turkish tobacco. In the plants in which this movement occurred, symptoms appeared on the basal graft in an average time of 8.4 days, 3.2 days later than they appeared on the inoculated grafts of the same plants, and 3 days later than symptoms appeared on directly inoculated basal grafts. If it is considered that this interval represents the time for the virus to move through the *N. glauca* stem from one graft to the other, the rate of movement was 36 inches in about 72 to 77 hours, or about one-half inch per hour.

As in similar experiments with Turkish tobacco (4), the acropetal movement was much delayed. The minimum time for the appearance of symptoms on a top graft, in the plants inoculated on the basal graft, was 32 days. In 7 of the 10 plants tested, no symptoms were evident on the top graft in periods ranging from 224 to 252 days.

When the experiment was discontinued, the stems of the seven plants that had produced no symptoms of mosaic on the top graft were severed one-half inch above the point of contact with the basal graft. To determine how far the virus had moved acropetally in the time interval allowed for movement, the stems were cut into 6-inch segments and juice was pressed from each segment and used to inoculate healthy Turkish tobacco plants. A record of the results of the tests from each of these segments is shown in table 2. It is evident from these results that the virus moved toward the top in each of the plants, but the shortness of the distance through which the virus

was able to travel is very striking, especially in those plants in which it was unable to move past the first 6 inches of stem. It is evident that under these conditions movement counter to the direction of predominant food flow was difficult.

TABLE 2.—Movement of virus from basal grafts of Turkish tobacco upward through stems of plants of *Nicotiana glauca*

Plant No.	Period from inoculation of basal graft to testing stem	Presence or absence of virus in indicated 6-inch segment of stem counting from base to top ¹						
		First	Second	Third	Fourth	Fifth	Sixth	Seventh ²
	<i>Days</i>							
1.	224	+	—	—	—	—	—	—
2.	225	+	+	—	—	—	—	—
3.	225	+	—	—	—	—	—	—
4.	225	+	+	+	—	—	—	—
5.	250	+	+	+	+	+	—	—
6.	251	+	+	—	—	—	—	—
7.	252	+	+	+	—	—	—	—

¹ Plus and minus signs indicate virus present or absent, respectively.

² 6-inch segment of stem from Turkish tobacco graft at top of plant.

MOVEMENT OUT OF ROOTS

The slow movement of virus acropetally from inoculated basal suckers in Turkish tobacco raised the question whether or not virus would move from the root system in plants under normal conditions of growth. Mulvania (15) found that when roots of potted plants were inoculated with the virus of tobacco mosaic no symptoms developed in the tops during the period of observation. Johnson (11) obtained similar results with potted plants. Lehman (12), in field tests, found that only a very small percentage of plants set in virus-infested soil developed symptoms of mosaic before topping time. Following topping, there was a marked increase in percentage of plants showing symptoms. All of these investigators concluded that infection of roots was rare or did not occur.

However, Price (16), in more recent work, concluded that roots of plants of *Nicotiana glutinosa* and Turkish tobacco were susceptible to infection. He was able to obtain relatively high concentrations of virus from roots of some of the inoculated plants 21 days after inoculation although the plants showed no symptoms.

In view of the apparent relationship between virus movement and food translocation it seems possible that these failures to obtain symptoms in root-inoculated plants may have been due to inability of the virus to move from the root system into the top, where symptoms could be expressed.

In order to test this theory, experiments of the following type were made. Small Turkish tobacco plants were placed in 3-inch pots and allowed to grow until they were approximately 6 inches tall. At this stage of development, two or three of the lowest leaves were removed from each plant in order to reduce the chances of accidental infection in later manipulations and the plants were removed from the pots. The root systems had become somewhat pot-bound, and each ball of soil was covered with a fairly compact mass of roots. The roots on the lower half of each ball of soil were inoculated by

rubbing them with a cloth saturated with inoculum. The plants were then placed in 8-inch pots. At the same time other plants were transferred directly, without root inoculation, to 8-inch pots and inoculated on the lowest remaining leaf.

The plants were allowed to develop normally under greenhouse conditions, but care was taken to avoid having any of the leaves come in contact with the soil or with the pots. At 10-day intervals for 40 days, five plants on which root inoculations had been made were cut back, leaving a stem about 2 inches long from which, on most plants, lateral buds soon developed into shoots.

The severed tops were defoliated and the stems were cut into 6-inch segments, which were planted in sand to determine by subsequent growth whether the virus was present at the time the tops were removed. The results of tests of the stem segments of the plants of one experiment are shown in table 3.

TABLE 3.—*Movement of virus out of the roots of Turkish tobacco plants before and after removal of tops*

Plant No.	Treatment after inoculation	Period for appearance of symptoms, counting from —		Presence or absence of virus in indicated 6-inch segment of stem counting from base to top ²					
		Removal of top ¹	Inoculation	First	Second	Third	Fourth	Fifth	Sixth
		<i>Days</i>	<i>Days</i>						
1	Top removed after 10 days.	11	21	+	—	—	—	—	—
2		10	20	—	—	—	—	—	—
3		21	31	—	—	—	—	—	—
4		10	20	—	—	—	—	—	—
5		9	19	—	—	—	—	—	—
6	Top removed after 20 days.	6	26	+	+	+	+	—	—
7		6	26	—	—	—	—	—	—
8		7	27	—	—	—	—	—	—
9		7	27	—	—	—	—	—	—
10		7	27	—	—	—	—	—	—
11	Top removed after 30 days.	6	36	—	—	—	—	—	—
12		6	36	—	—	—	—	—	—
13		7	37	—	—	—	—	—	—
14		—1	29	+	+	+	+	+	+
15		7	37	+	+	+	+	+	+
16	Top removed after 40 days.	—9	31	+	+	+	+	+	+
17		8	48	+	—	—	—	—	—
18		7	47	—	—	—	—	—	—
19		—5	35	+	+	+	+	+	+
20		7	47	—	—	—	—	—	—
21	Top not removed.	—	67	—	—	—	—	—	—
22		—	35	—	—	—	—	—	—
23		—	57	—	—	—	—	—	—
24		—	63	—	—	—	—	—	—
25		—	13	—	—	—	—	—	—
26	Top not removed; inoculated on lowest leaf.	—	6	—	—	—	—	—	—
27		—	8	—	—	—	—	—	—
28		—	6	—	—	—	—	—	—
29		—	8	—	—	—	—	—	—
30		—	7	—	—	—	—	—	—

¹ A minus sign preceding a number shows that symptoms appeared on the top of the plant the indicated number of days before the top was removed.

² Plus and minus signs indicate virus present or absent, respectively.

A record was kept of the time of appearance of symptoms on all of the inoculated plants. A high percentage of infection was obtained in all lots of plants in which the roots were inoculated. Symptoms, however, were very much delayed, and removal of tops had a decided influence on the time of appearance of symptoms. Table 3 shows in plants 6. to 20, from which the tops were removed 20 to 40 days

after inoculation, that symptoms of mosaic appeared in the new growth of the lateral buds in 6 to 8 days after the tops were removed. In plants 1 to 5, from which the tops were removed 10 days after inoculation, the longer periods required for appearance of symptoms probably were due to very slow growth following removal of the tops.

It may be noted that the plants that were inoculated on the lowest remaining leaf produced symptoms in 6 to 8 days after inoculation, showing that the virus was able to move to the top of the plant from points near the base of the stems in a relatively short time. However, assuming correlation between virus movement and food transport, this movement would be expected if frequent reversals of food movement occurred in the stem. It seems probable that considerable food storage may occur in the base of the stem and even in the larger roots and that at times there would be acropetal movement of these materials. It would be expected, also, that in the distal portion of the root system of a normal plant movement would be unidirectional and toward the root tips. Virus would have difficulty in moving out of small roots under such conditions. Removal of tops, however, might extend the zone in the root system in which movement of foods acropetally could take place. This would account for the rapid movement of virus out of the inoculated roots following removal of the tops of the plants.

EFFECT OF DEFOLIATION ON MOVEMENT

Previous studies (4) have shown that the virus of curly top moved upward at a relatively rapid rate in defoliated stems of *Nicotiana glauca* but that upward movement was greatly retarded in foliated stems. Tests were made in an effort to determine the effect of defoliation on the movement of the virus of tobacco mosaic in plants of *N. tabacum*, Turkish variety, and experiments with *N. glauca*, similar to those in which the movement of the virus of curly top was studied, were made with the virus of tobacco mosaic.

EXPERIMENTS WITH NICOTIANA TABACUM

Potted plants of Turkish tobacco 24 to 30 inches tall were bent so that their stems approached a horizontal position. When a selected basal sucker was about 2 inches tall it was inoculated and the main stem was immediately defoliated and pruned to a length of 30 to 36 inches. At intervals of 2 days for 18 days, beginning with the fourth day after inoculation, stems were cut into 3-inch segments and tested for the presence of virus by using extracted juice from each segment to inoculate healthy plants of Turkish tobacco. Five plants were tested at each time interval, and five foliated plants were tested at the end of the experiment as a check on the effect of defoliation on the acropetal movement of the virus.

The extent of the acropetal movement of the virus from the inoculated suckers through the defoliated and foliated stems, as indicated by the tests of stem segments, is shown in table 4. These results show that acropetal movement was relatively fast in defoliated stems and that by the twelfth day the virus had moved the full length of the stem of each plant. Results from the 14-, 16-, and 18-day tests were the same as those from the 12-day tests, and for this reason they are not shown in table 4. The results obtained with the foliated

check plant show that with normal foliage the acropetal movement was much retarded, since in 18 days the virus had not traveled more than 9 inches and in two of the plants it apparently had not entered the first basal 3-inch segment.

TABLE 4.—*Effect of defoliation on acropetal movement of virus in stems of Nicotiana tabacum*

Plant No.	Period from inoculation and defoliation to testing	Presence or absence of virus in indicated 3-inch segment counting from base to top ¹											
		First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth	Eleventh	Twelfth
	Days												
1	4	—	—	—	—	—	—	—	—	—	—	—	—
2		—	—	—	—	—	—	—	—	—	—	—	—
3		—	—	—	—	—	—	—	—	—	—	—	—
4		—	—	—	—	—	—	—	—	—	—	—	—
5	6	—	—	—	—	—	—	—	—	—	—	—	—
6		—	—	—	—	—	—	—	—	—	—	—	—
7		—	—	—	—	—	—	—	—	—	—	—	—
8		—	—	—	—	—	—	—	—	—	—	—	—
9	8	—	—	—	—	—	—	—	—	—	—	—	—
10		—	—	—	—	—	—	—	—	—	—	—	—
11		—	—	—	—	—	—	—	—	—	—	—	—
12		—	—	—	—	—	—	—	—	—	—	—	—
13	10	—	—	—	—	—	—	—	—	—	—	—	—
14		—	—	—	—	—	—	—	—	—	—	—	—
15		—	—	—	—	—	—	—	—	—	—	—	—
16		—	—	—	—	—	—	—	—	—	—	—	—
17	12	—	—	—	—	—	—	—	—	—	—	—	—
18		—	—	—	—	—	—	—	—	—	—	—	—
19		—	—	—	—	—	—	—	—	—	—	—	—
20		—	—	—	—	—	—	—	—	—	—	—	—
21	14	—	—	—	—	—	—	—	—	—	—	—	—
22		—	—	—	—	—	—	—	—	—	—	—	—
23		—	—	—	—	—	—	—	—	—	—	—	—
24		—	—	—	—	—	—	—	—	—	—	—	—
25	16	—	—	—	—	—	—	—	—	—	—	—	—
26		—	—	—	—	—	—	—	—	—	—	—	—
27		—	—	—	—	—	—	—	—	—	—	—	—
28		—	—	—	—	—	—	—	—	—	—	—	—
29	18 (Check ²)	—	—	—	—	—	—	—	—	—	—	—	—
30		—	—	—	—	—	—	—	—	—	—	—	—

¹ Plus and minus signs indicate virus present or absent, respectively.

² Plants not defoliated.

EXPERIMENTS WITH NICOTIANA GLAUCA

Plants with stems 36 inches long and with grafts of Turkish tobacco at the top and base arranged as described in previous experiments with *Nicotiana glauca* were used in further tests of the effects of defoliation on upward movement of virus in stems. When the shoots from the basal grafts of these plants were about 12 inches long, they were inoculated and the plants were left undisturbed for 12 days. At the end of this period, all the inoculated shoots showed distinct symptoms of mosaic and all the grafts at the tops of the plants were normal. After the 12-day period, five of the stems were cut into 6-inch segments and incubated in a moist chamber at room temperature for 24 hours, after which expressed juice from each segment was used to inoculate healthy Turkish tobacco plants. The results from these inoculations indicate the average extent of upward invasion during the first 12 days following the original inoculation (fig. 1, check *a*). All of the other plants, except five (fig. 1, check *b*), that were retained as controls, and tested at the end of the experiment, were defoliated

except for the basal inoculated graft. At intervals of 2 days for a period of 18 days, stems were cut into 6-inch segments and tested for virus as already described (fig. 1). On the eighteenth day, the remaining foliated check plants were cut into 6-inch segments and tested for virus. The average distances of virus movement, in inches, in the defoliated stems from the basal inoculated grafts, in comparison with virus movement in the foliated checks *a* and *b*, are shown in figure 1.

Acropetal movement of virus started in less than 4 days after defoliation of the main stem and progressed rapidly until the entire stem of each of the plants was invaded in 12 days or longer. Figure 2 shows a defoliated plant in which the virus moved to the top and produced distinct symptoms of mosaic within 12 days. The results of the tests of stem segments of the two lots of plants that were not defoliated show that in these plants, growing under more or less normal conditions, relatively little movement acropetally occurred during the course of the experiment. These results

demonstrate conclusively that the acropetal movement of virus in the defoliated plants was a direct response to defoliation.

These results reemphasize the fact that under certain conditions acropetal movement of virus into leafy tops of plants, presumably counter to the major movement of elaborated food materials, is difficult and usually very slow as compared to movement in the reverse direction. They show further that, when the carbohydrate-synthesizing areas are removed or reduced so that a food deficit results, movement into the defoliated parts is greatly accelerated.

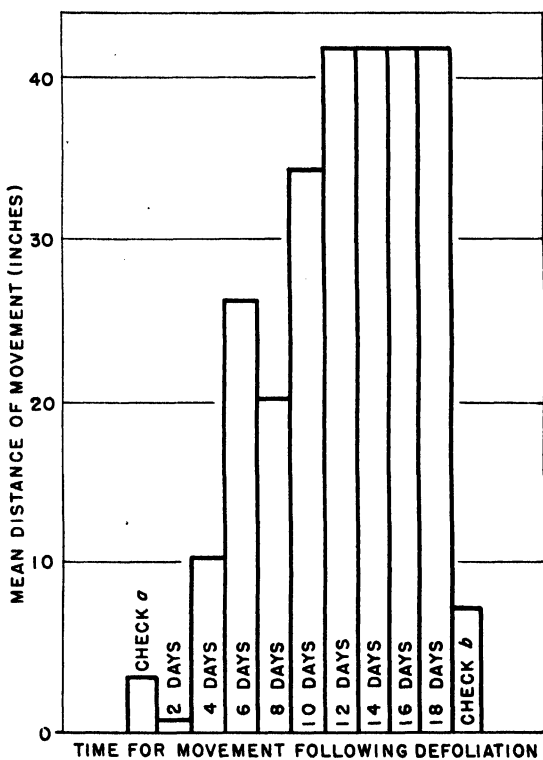


FIGURE 1.—Effect of defoliation on movement of tobacco mosaic virus from infected basal grafts of Turkish tobacco upward through defoliated stems of *Nicotiana glauca* toward top grafts of Turkish tobacco. Results are given as 5-plant averages of data obtained from the tests of 6-inch stem segments taken, at intervals of 2 days, for 18 days after the initial 12-day period allowed for establishment of the virus in the inoculated basal graft. Checks *a* and *b* were not defoliated.

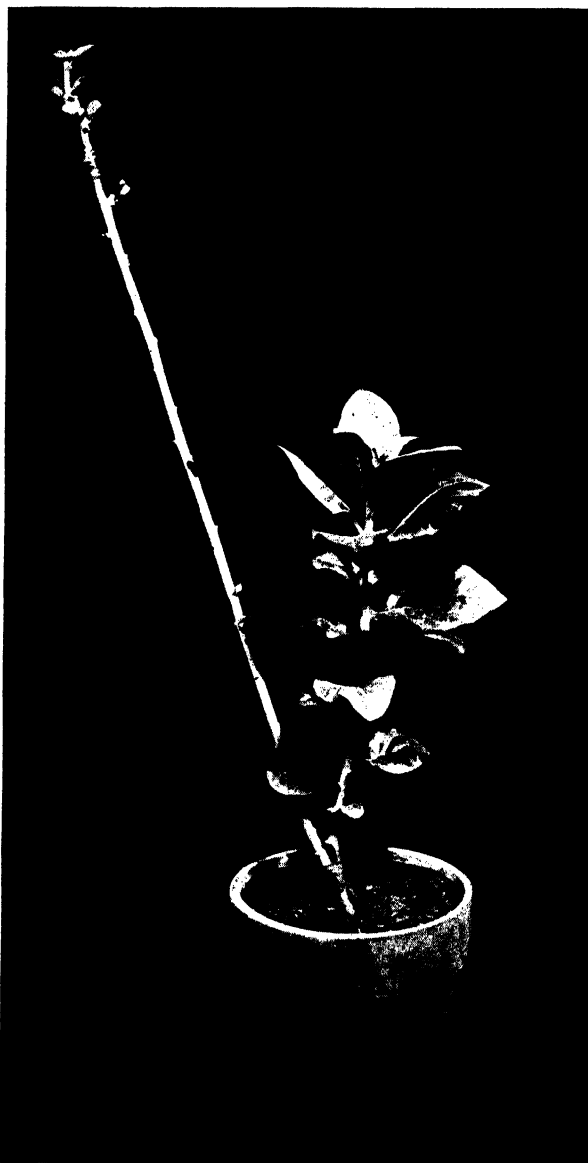


FIGURE 2.—Effect of defoliation on upward movement of virus. This plant of *Nicotiana glauca* was inoculated on the basal graft of Turkish tobacco; 12 days later all foliage was removed from the main stem. Photographed 12 days after defoliation. Symptoms of mosaic are evident on the rolled leaves of the new growth of the top graft of Turkish tobacco.

When the results shown in figure 1 are compared with the results (4, *fig. 2*) obtained in the study of the upward movement of the curly top virus in the same type of plant under similar conditions, it seems probable that, under the conditions of the tests, movements of the two

viruses were similar and were influenced by the same factors operating approximately equally in each plant.

EFFECT OF DARKNESS ON MOVEMENT

Turkish tobacco plants with main stems in a horizontal position and with basal suckers similar to those already described were used in experiments to test the influence of darkness on acropetal movement of virus. The main stems of the selected plants were 30 to 36 inches long at the time the tests were started.

In the first tests, the main stems of the plants were placed in a dark box but the basal suckers were exposed to the normal light conditions of the greenhouse throughout the tests. The basal suckers were inoculated at the time the main stems were placed in the dark. At intervals of 5 days for 20 days following inoculation, plants were removed from the dark and the main stems were defoliated and cut into 6-inch segments. Juice was pressed from each segment and used to inoculate healthy Turkish tobacco plants to determine the presence or absence of virus.

Movement of the virus in an acropetal direction from the inoculated sucker was slow at first and apparently little movement occurred during the first 5 days (table 5). Movement was more marked during the second and third 5-day periods, and after 20 days virus was found in all the segments of each of the stems of the plants of this series.

TABLE 5. — *Movement of virus in an acropetal direction in darkened horizontally placed main stems of Turkish tobacco plants, from basal upright shoots in the light*

Plant No.	Period from inoculation to testing	Presence or absence of virus in indicated 6-inch segment of stems after indicated period in dark, counting from base to top ¹					
		First	Second	Third	Fourth	Fifth	Sixth
	<i>Days</i>						
1	5	+	—	—	—	—	—
2		+	—	—	—	—	—
3		—	—	—	—	—	—
4		—	—	—	—	—	—
5		—	—	—	—	—	—
6	10	+	—	—	—	—	—
7		+	+	+	+	+	+
8		+	+	+	—	—	—
9		+	+	—	—	—	—
10		+	—	—	—	—	—
11	15	+	+	+	+	+	+
12		+	+	+	+	+	+
13		+	+	+	+	+	+
14		+	+	+	+	+	+
15		+	+	+	+	+	+
16	20	+	+	+	+	+	+
17		+	+	+	+	+	+
18		+	+	+	+	+	+
19		+	+	+	+	+	+
20		+	+	+	+	+	+

¹ Plus and minus signs indicate presence or absence of virus, respectively.

Although comparison of these results with those obtained with similar plants growing under more nearly normal conditions of illumination (table 1) indicates that darkness had a marked effect in inducing acropetal movement, the response in virus movement seems less striking than that previously obtained (4) with the virus of curly

top in sugar beet. This difference may be associated in part with yellowing and dying of darkened tobacco leaves, which may have resulted in an outward movement of materials from these leaves during the first few days they were in the dark, and this in turn may have tended to delay the movement of materials, including virus, from the basal parts of the plants.



FIGURE 3.—Plant of *Nicotiana tabacum* inoculated on a small leaf at the top of the main shoot immediately after it was placed in the dark. The basal upright shoot was exposed to the light. The plant, photographed 40 days after inoculation, shows the new growth produced in the dark, the dead leaves on the older part of the plant, and the growth produced by the basal sucker during the period of the experiment. The virus did not pass out of the main shoot of this plant in a period of 40 days in the dark.

In a second experiment, the main shoots of plants similar to those used in the experiment just described were placed in a dark box and the basal suckers were exposed to normal light conditions. At the time the shoots were placed in the dark, one of the small leaves at the top of each darkened shoot was inoculated. Check plants were used in which all parts were in the light.

The plants in the dark and the check plants were watched for the appearance of symptoms on the tops of the main shoots and on the basal suckers. Symptoms appeared on the inoculated tops of the check plants in an average period of 5.2 days and on the basal suckers in an average period of 7.8 days.

Owing to the etiolated condition of the tops of the shoots in the dark, symptoms were not distinguished on the inoculated parts of these plants. The

minimum time for the appearance of symptoms on a basal sucker was 29 days, and on 7 of 10 plants tested no symptoms were evident on the basal suckers after 40 days. At the end of the 40-day period, it was found that all of the darkened leaves that had normal green color when placed in the dark box had died but that from 15 to 24 inches of new

growth had been produced during the time the shoots were in the dark. Figure 3 shows one of these plants at the time it was removed from the dark.

Upon removal from the dark box after a period of 40 days, the main stems were defoliated and cut into 5-inch segments. Juice was pressed from these segments and used to inoculate healthy Turkish tobacco plants to determine the extent of virus spread from the points of inoculation during the period of test.

The distribution of virus in 7 of the 10 plants used in the experiment is shown in table 6. The results show that in these plants the virus was present in all of the etiolated segments but had moved to the basal sucker in only one plant. Symptoms appeared on the basal sucker of this plant a few days after the main stem was removed. The parts of the remaining 6 plants from which the main stems had been removed were kept for several months. All remained free of symptoms. Thus, in 6 of the 10 plants the virus was unable to pass basipetally out of the darkened shoots in a period of 40 days.

TABLE 6.—*Distribution of virus in inoculated main stems of plants of Turkish tobacco after they had been in the dark for 40 days*

[Each plant had an upright basal shoot that was kept under normal light conditions]

Plant No.	Presence or absence of virus in indicated 5-inch segment of stem, counting from base to top ¹								
	First	Second	Third	Fourth	Fifth	Sixth ²	Seventh ³	Eighth ³	Ninth ³
1	+	+	+	+	+	+	+	+	+
2	—	—	—	—	—	—	—	—	—
3	—	+	—	+	+	+	+	+	+
4	—	—	+	+	+	+	+	+	+
5	—	—	—	+	+	+	+	+	+
6	—	—	—	+	+	+	+	+	+
7	—	—	—	+	+	+	+	+	+

¹ Plus and minus signs indicate virus present or absent, respectively.

² Inoculated segment.

³ Etiolated segments produced in the dark; the ninth segment ranged from 4 to 9 inches long, depending on the plant.

EFFECT OF SEED PRODUCTION ON MOVEMENT

Vickery et al. (18) have shown that at the time the tobacco plant fruits there is an appreciable movement of carbohydrates and other materials from the mature leaves and from the root system into the flower parts. It seems likely that this movement may be extensive enough to cause a more or less uninterrupted movement of materials toward the fruiting parts during the time seeds are being produced. Periodic directional reversal of flow of materials, therefore, may occur only sparingly or not at all at such times in these plants. If this is true and if virus movement is correlated with food flow, it follows naturally that acropetal movement of virus in fruiting plants should be more rapid than in nonfruiting plants and that basipetal movement should be less rapid.

Turkish tobacco plants with horizontally placed stems and with basal suckers as already described were used to test this theory. Four lots of plants were prepared. In the first, the basal sucker was inoculated before the plants had produced blossom buds. To keep the plants in a vegetative condition so far as possible, all fruiting structures were removed before any blossoms opened. Figure 4

shows one of the plants of this lot. In the second, third, and fourth lots the basal suckers were inoculated, respectively, when the first blossom opened, when the first seed pod was full size, and when the most mature seed pod was beginning to turn brown. In all four lots of plants, however, the bending of the main stem was timed so that the basal suckers were less than 6 inches long when they were inoculated.



FIGURE 4.—Type of plant of *Nicotiana tabacum* used to study the acropetal movement of virus in a main stem of tobacco. The basal sucker was inoculated when it was about 2 inches tall and blossom buds were removed from the main stem before any blossoms opened. Photographed 30 days after the basal sucker was inoculated.

lot. In the three lots of plants that were allowed to fruit the virus moved into the tops of all of the plants in periods of 5 to 15 days after appreciable acropetal movement had started.

These results show that conditions are unfavorable for acropetal movement of virus in tobacco plants of the kind tested prior to the time of fruiting, but that there is a period at the time of seed production during which conditions are peculiarly favorable for movement of virus in the direction of the fruiting parts. This period falls within the time during which the plants are transporting appreciable quan-

The plants of the second, third, and fourth lots were allowed to fruit normally. At intervals of 5 days, following the inoculation of the basal suckers, five plants of each lot were cut into 6-inch segments and each segment was planted in sand in a separate pot. Subsequent growth indicated presence or absence of virus in the segment and showed the extent of invasion of the main stems of the plants in the allotted time intervals.

In these plants the acropetal movement of the virus in the main stem from the inoculated sucker was influenced to a marked degree by seed production. The extent of this influence is illustrated in figure 5. In the plants that were not allowed to blossom the virus failed to reach the top of the main stems in a period of 40 days except in one of the plants in the 30-day

tities of organic materials to the fruiting parts for the production of seeds.

Since fruiting was correlated with a marked increase in rate of movement of virus from the base of the plant toward the fruiting area, it would seem to follow that movement in the reverse direction, or basipetally, would be less rapid in fruiting plants than in plants in a vegetative condition if virus movement is influenced by the factors responsible for food translocation.

In an experiment planned to test this hypothesis, plants of Turkish tobacco were bent to a horizontal position as in other experiments.

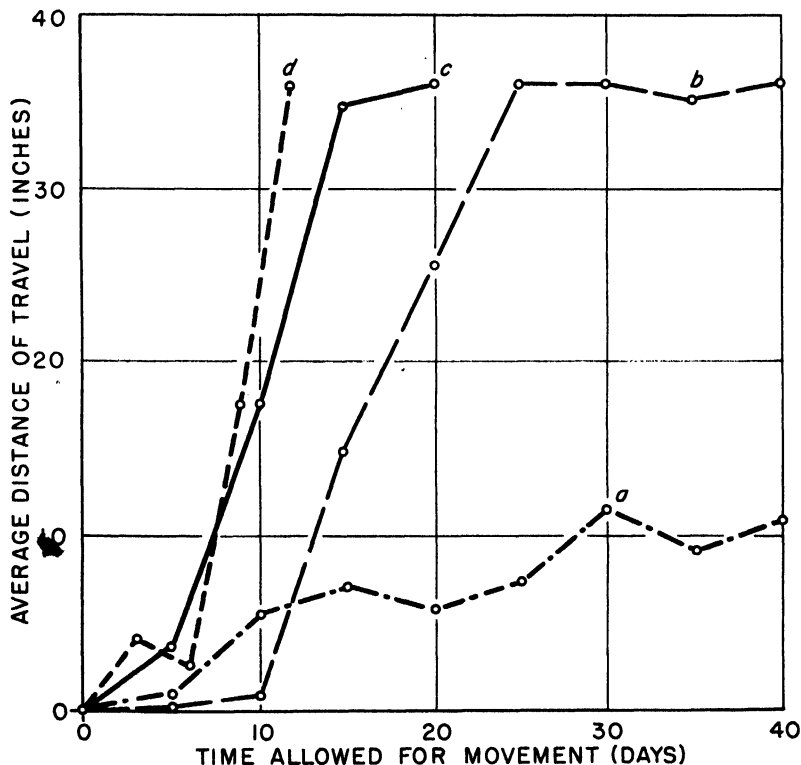


FIGURE 5.—Acropetal movement of the virus of tobacco mosaic in the main stems of plants of *Nicotiana tabacum* into which the virus was introduced through basal suckers when the main stems were in the following stages of development: a, Vegetative, not allowed to blossom; b, first blossom open; c, most mature seed pod full size; d, most mature seed pod brown.

When the seed pods of the main stem began to turn brown, leaves of the inflorescence were inoculated. At 5-day intervals for 20 days, stems were severed one-half inch above the point of attachment of the basal sucker, defoliated, cut into 6-inch segments, and tested for the presence of virus by using expressed juice from each segment to inoculate healthy Turkish tobacco plants.

An equal number of check plants were used. These, however, were younger, and when leaves at the distal end of the main stems were inoculated no blossom buds were evident. Stems were severed daily

for 5 days after inoculation, and 6-inch segments were tested for the presence of virus by the method used for the lots of older plants.

The difference in rates of basipetal movement of virus in these two types of plants is striking as shown in table 7, where the results of tests of the segments of each plant are recorded. Movement of the virus in the direction of the root system was very rapid in the vegetative plants, as was expected. In two of these plants the virus moved a distance of 36 inches in 3 days, and in all of the plants tested it moved a distance of 36 inches or more in 4 days. In these plants the virus had either moved the full distance to the roots in the time interval selected or it had not moved out of the inoculated segment.

In the fruiting plants, movement was much delayed and in one plant the virus did not move out of the inoculated segment in 20 days. In five of the fruiting plants the virus was intercepted in its movement toward the root even though the interval of test was 5 days. This suggests that throughout the entire length of the stems basipetal movement may have been slow as compared with that in stems of vegetative plants, where the virus was not intercepted in its movement toward the roots in any instance, although the interval of test was only 1 day.

TABLE 7.—*Influence of stage of maturity of Turkish tobacco plants on basipetal movement of virus*

Stage of development of inoculated plant	Period from inoculation to test for virus	Plant No.	Basal sucker ¹	Presence or absence of virus in indicated 6-inch segment counting from base to top ¹					
				First	Second	Third	Fourth	Fifth	Sixth ²
Oldest seed pods beginning to turn brown.	<i>Days</i>								
	5	1	—	—	—	—	+	—	+
		2	—	—	—	—	—	—	+
		3	—	—	—	—	—	—	+
		4	—	—	—	—	—	—	+
		5	—	—	—	—	—	—	+
	10	6	—	—	—	—	—	—	+
		7	—	—	—	—	—	—	+
		8	+	+	+	+	+	+	+
		9	—	—	—	—	—	—	+
		10	—	—	—	—	—	—	+
	15	11	—	—	—	—	—	+	+
		12	—	—	—	—	—	+	+
		13	+	+	+	+	+	+	+
		14	—	—	—	—	+	+	+
		15	—	—	—	—	+	+	+
	20	16	+	+	+	+	+	+	+
		17	—	—	—	—	—	—	+
		18	+	+	+	+	+	+	+
		19	+	+	+	+	+	+	+
		20	+	+	+	+	+	+	+
Plants wholly vegetative, blossom buds not evident.	2	21	—	—	—	—	—	—	+
		22	—	—	—	—	—	—	+
		23	—	—	—	—	—	—	+
		24	—	—	—	—	—	—	+
		25	—	—	—	—	—	—	+
	3	26	—	—	—	—	—	—	+
		27	—	—	—	—	—	—	+
		28	+	+	+	+	+	+	+
		29	—	—	—	—	—	—	+
		30	+	+	+	+	+	+	+
	4	31	+	+	+	+	+	+	+
		32	+	+	+	+	+	+	+
		33	+	+	+	+	+	+	+
		34	+	+	+	+	+	+	+
		35	+	+	+	+	+	+	+

¹ Plus and minus signs indicate virus present or absent, respectively.

² Inoculated segment.

RESISTANCE OF DIFFERENT TISSUES OF STEMS TO
PASSAGE OF VIRUS

Previous experiments (3) have shown that the virus of curly top of sugar beet did not move through portions of stems of *Nicotiana tabacum* and *N. glauca* in which the phloem continuity was broken by ringing. However, the virus was able to move in both the external and internal phloem and to pass from one type to the other through the union of the two in the leaf traces.

The virus of curly top shows evidence of being limited to the phloem, and for this reason probably would not be expected to pass through such structures as medullary rays, wood parenchyma, or other types of parenchymatous tissues. The virus of tobacco mosaic, however, occurs in much of the tissue outside of the phloem and probably multiplies and reaches high concentrations in parenchyma of the common varieties of commercial tobaccos. With these concepts in mind, tests were made to determine the effect of various types of rings in stems of *Nicotiana tabacum* and *N. glauca* on the passage of virus.

EXPERIMENTS WITH NICOTIANA TABACUM

Potted plants of *Nicotiana tabacum* were allowed to grow until they reached the blossom stage, when they were pruned back to a height of about 18 inches. Internal and external rings were then placed in the stem below the second bud counting from the top of the stem. The external ring was made by removing a ring of bark and cambium approximately 2 mm. wide. The internal ring was made by removing a part of the stem by means of a small cork borer and scraping out a ring of pith and internal phloem.

After the rings were made the plants were allowed to stand for 3 or 4 days, when the rings were again scraped and the holes for the internal rings were filled with paraffin having a melting point of 60° C. Leaves on parts above the rings were then inoculated and the plants were observed for the appearance of symptoms on parts above and below the rings.

The position of the rings and the results of the tests are shown in table 8. As indicated, the rings were placed in positions to determine whether the virus was able to move through (1) internal phloem, (2) external phloem, (3) vertically through the woody cylinder, (4) from external to internal phloem through the woody cylinder, (5) from internal to external phloem through the woody cylinder, (6) from internal to external phloem through the phloem connections in the leaf traces, and (7) from external to internal phloem through connections in the leaf traces.

There was no appreciable delay in passage of virus through rings in which there was uninterrupted phloem tissue. Virus passed radially through the woody cylinder in the absence of phloem connections, but apparently passage was delayed an average period of 8 days or more. It seems evident also that the virus was able to travel vertically in the woody cylinder, but travel was rather slow and movement of a distance of little more than 2 mm. apparently required an average time of more than 20 days as indicated by the delay in appearance of symptoms on shoots across the rings. However, it is possible that a part of this apparent delay was caused by the time required for the

virus to move into the tops of the indicator shoots, which had grown considerably during the period of the experiment.

TABLE 8.—Effect of different types of rings in stems of *Nicotiana tabacum* on the movement of virus through the ringed parts

Position of rings	Plants tested	Average period for appearance of symptoms on shoots	
		Above rings	Below rings
	Number	Days	Days
External ring only.....	5	4.8	6.1
Internal ring only.....	5	4.4	5.8
External and internal rings at same level.....	12	6.2	29.5
External ring 1 inch above internal, both in internode.....	6	5.0	14.0
Internal ring 1 inch above external, both in internode.....	5	6.0	13.8
External ring 1 inch above internal, node between.....	5	5.4	6.6
Internal ring 1 inch above external, node between.....	5	5.4	6.0

This delay in virus passage may account for the results obtained by Matsumoto and Somazawa (13) in which they found that, although the virus of tobacco mosaic occurred in the woody cylinder of tobacco stems, it apparently did not pass portions of the stem from which both internal and external phloem had been removed. If tests for presence of virus were made shortly after symptoms appeared on the inoculated shoots, as may be inferred, it is probable that not enough time was allowed to permit passage of virus through the ringed parts of the stems.

EXPERIMENTS WITH NICOTIANA GLAUCA

An experiment similar to that just described was made with *Nicotiana glauca*, instead of Turkish tobacco, to test the movement of virus through different stem tissues. Plants having single stems were pruned to a height of about 24 inches, and healthy scions of Turkish tobacco were grafted into the base and top of each stem. When the grafts were well established, rings of the same type as those used in Turkish tobacco in the experiment already described were placed in the stems of *N. glauca* a short distance below the top graft. After the rings were made, the top graft of each plant was inoculated and the plants were watched for the appearance of symptoms of mosaic on the grafts of Turkish tobacco above and below the rings.

As in experiments with *Nicotiana tabacum*, rings that did not destroy phloem continuity had no measurable effect on the movement of the virus through the stem. However, the results obtained from the plants in which phloem continuity was broken by the rings were decidedly different from those obtained with *N. tabacum*. In some of the plants (table 9) in which there were no phloem strands bridging the rings, symptoms appeared on the lower graft after considerable delay. Perhaps in these plants the virus passed the rings in the absence of phloem. However, it is recognized that in experiments of this type there is always danger of accidental infection, since healthy shoots of the very susceptible Turkish tobacco were growing in close proximity to infected parts.

TABLE 9.—Effect of different types of rings in the stem of *Nicotiana glauca* on passage of the virus of tobacco mosaic

Position of rings	Plants infected	Effect on plants in which virus passed rings		Effect on plants in which virus did not pass rings	
		Plants affected	Average period from inoculation to appearance of symptoms below ring	Plants affected	Average period from inoculation to discard of plants
	Number	Number	Days	Number	Days
No ring.....	5	5	7.8		
External ring only.....	5	5	8.6		
Internal ring only.....	5	5	6.4		
External and internal rings at same level.....	5	1	19.0	4	376
External ring 1 inch above internal, both in internode.....	5	3	104.5	2	390
Internal ring 1 inch above external, both in internode.....	4	3	46.3	1	327
External ring 1 inch above internal, node between.....	5	5	8.2		
Internal ring 1 inch above external, node between.....	5	5	7.8		

Regardless of what may have happened in the stems of the plants in which symptoms of mosaic appeared on both sides of the rings, it seems clear that at least some of the plants were able to restrict the virus to a certain portion below which phloem continuity was severed. In some of these plants the virus was held in the parts above rings for periods of several months. One of these plants is shown in figure 6. A more detailed view of another plant of this series is shown in figure 7.

At the time the plants in which the virus failed to pass the rings were discarded, transverse sections of the stems in the ringed portions were examined to determine whether living cells connected the parts above and below the rings. Where the rings were at the same level, the exposed inner and outer tissue enclosed a part that seemed similar to normal wood. Living wood parenchyma cells were present, some with chloroplasts. Where the rings were at different levels, the pith, wood, and bark appeared normal between the rings, and numerous living strands bridged the space between the inner and outer phloem. From these observations it was concluded that there were bridges of living tissue connecting the phloem of the upper infected part with the lower noninfected part in all of the plants in which the virus failed to pass the rings.

These results seem to justify the conclusion that in at least some of the plants of *Nicotiana glauca* the living parenchyma of the stem is able to act as a barrier to the passage of the virus of tobacco mosaic. In such stems both the medullary rays and the wood parenchyma are impermeable to the virus.

MOVEMENT OF THE VIRUS OF CUCUMBER MOSAIC THROUGH RINGS IN THE STEMS OF *NICOTIANA GLAUCA*

After it was found that the virus of tobacco mosaic was unable to move longitudinally or radially through the woody cylinder of certain plants of *Nicotiana glauca* in the rather long time interval allotted for movement, experiments with a second virus were started. The virus



FIGURE 6.—Restriction of virus movement in stems of *Nicotiana glauca* by rings that destroyed phloem continuity. Grafts of Turkish tobacco were placed at the top and base of the stem. External and internal rings (positions shown by arrows) were placed at different levels in an internode, and the top graft was inoculated September 18, 1936. The plant was photographed April 14, 1937. The mosaic symptoms in the top graft are in striking contrast to the normal growth from the basal graft, showing that the virus was unable to traverse the woody cylinder of the stem, vertically or radially.



FIGURE 7.—More detailed view of plant of same series as that shown in figure 6. Photographed 6 months after the top graft of Turkish tobacco was inoculated with the virus of tobacco mosaic. The virus did not pass the rings (positions shown by arrows) in the stem of *Nicotiana glauca* in a period of 6 months, as indicated by the healthy foliage at the top of a shoot from the basal graft of Turkish tobacco shown in the lower part of the figure.

selected for these tests occurs in many of the plants of *N. glauca* growing wild in the vicinity of Riverside, Calif. This virus is able to attack a number of plant species and causes very distinct mottling on *N. glauca* and on Turkish tobacco, although on the latter mottling is much less pronounced than that caused by the virus of tobacco mosaic. Its properties and host range, so far as determined, indicate that it is a strain of the virus of cucumber mosaic common in the United States.

Test plants were prepared as already described in the experiments with the virus of tobacco mosaic. Stems of plants of *Nicotiana glauca* were cut back to a height of about 24 inches and grafts of Turkish tobacco were placed at the top and base. After the grafts were well established, rings were placed in the stems of the *N. glauca* plants about 1 to 2 inches below the top graft. These rings are listed in table 10 and were designed to determine (1) movement through the normal uninjured stem, (2) longitudinal movement through the woody cylinder, (3) radial movement through the woody cylinder, and (4) movement through the phloem of the leaf traces. After the rings were made, the top graft of each plant was inoculated.

TABLE 10.—Effect of different types of rings in the stem of *Nicotiana glauca* on movement of the virus of cucumber mosaic

Position of rings	Plants tested	Average time for appearance of symptoms on grafts	
		Above rings	Below rings
	Number	Days	Days
No ring	5	20.2	24.6
External and internal ring at same level	13	19.3	43.3
External and internal rings at different levels, both in internode	28	15.5	39.3
External and internal rings at different levels, node between the two rings	5	19.8	20.4

The symptoms produced in these inoculated plants varied considerably, especially on Turkish tobacco. However, they were distinct enough to be detected readily under favorable conditions. The incubation period of the virus was also subject to much variation in different plants and was much longer than that of the virus of tobacco mosaic in Turkish tobacco plants used in earlier experiments.

Average periods for the appearance of symptoms on the inoculated parts and on the parts below the rings are shown in table 10. In these tests the virus moved through the ringed portions of the stems regardless of the presence or absence of phloem connections. Rings that did not sever phloem continuity had no detectable effect on the rate of virus passage, whereas rings that severed phloem continuity caused marked delays in the time of appearance of symptoms on the lower grafts. This indicates a period of slow movement through the woody cylinder.

Another series of plants of *Nicotiana glauca* with top and basal grafts of Turkish tobacco was prepared for study of the effect of rings on passage of the viruses of tobacco mosaic and cucumber mosaic following simultaneous introduction of the two viruses into parts above double rings. After the grafts of these plants were well established, external and internal rings were placed at different levels in an inter-



FIGURE 8.—Plant of *Nicotiana glauca* with top and basal grafts of Turkish tobacco and rings (positions shown by arrows) breaking the continuity of the phloem in the stem of *N. glauca*. The viruses of tobacco mosaic and cucumber mosaic were introduced simultaneously into the top graft. The virus of cucumber mosaic moved through the ringed area and produced symptoms in a period of 37 days. The virus of tobacco mosaic failed to move through the ringed area in 100 days.

node in the stem of *N. glauca* about 2 inches below the lower end of the top graft. Each top graft was then inoculated with both viruses and all grafts were watched for the appearance of symptoms characteristic of those produced by each of the two viruses.

In all of the 20 plants tested, the virus of cucumber mosaic moved through the rings, and symptoms were evident on the foliage below the rings in an average period of 36 days. In 4 plants, symptoms of tobacco mosaic appeared on the lower grafts in an average period of 68 days.

No symptoms of tobacco mosaic were evident on the foliage of the lower grafts of the remaining plants in a period of 100 days, when the experiment was discontinued. Figure 8 shows one of these plants photographed 100 days after it was inoculated, and figure 9 shows

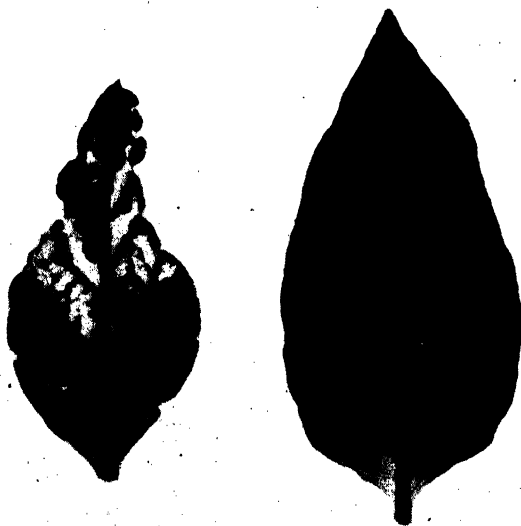


FIGURE 9.—Leaves of Turkish tobacco taken from the top and basal grafts on the plant of *Nicotiana glauca* (fig. 8) in the stem of which the virus of cucumber mosaic passed double rings that destroyed phloem continuity and the virus of tobacco mosaic failed to pass such rings: A, From top graft; B, from basal graft.

characteristic symptoms produced on leaves taken from the top and basal grafts at the time the plant was photographed.

At the end of the experiment, portions of the top and basal grafts of each plant were removed and dried to destroy the virus of cucumber mosaic. Extracts were prepared from the dried material and used to inoculate healthy tobacco plants. No infection was obtained from extracts from material taken from the basal grafts that did not show symptoms of tobacco mosaic.

These results show that certain plants of *Nicotiana glauca* separated the virus of cucumber mosaic from the virus of tobacco mosaic by virtue of the fact that the former moved through the woody cylinder

of *N. glauca* in the absence of phloem, under conditions which caused the restriction of the virus of tobacco mosaic to the inoculated parts above the rings.

DISCUSSION

In connection with the general subject of factors involved in the movement of viruses in plants, a comparison of the movement of the virus of tobacco mosaic in *Nicotiana tabacum*, Turkish variety, and in *N. glauca* with the movement of the virus of curly top (4) in these two species of *Nicotiana* is of interest.

The virus of curly top probably is closely limited to the phloem, but in the common varieties of tobacco the virus of mosaic evidently occurs in both phloem and parenchyma. Certain differences in the ability of two such viruses to invade plants would be expected to result from these different tissue relationships. The differences that appear most evident in the results obtained involve ability of the virus of mosaic to move through rings in the stems of plants of Turkish tobacco in the absence of continuous phloem paths, and in an apparently greater tendency on the part of the virus of mosaic to move in directions opposite food transport under certain conditions.

Before considering these differences further it should be emphasized that there is evidence of two distinct types of virus movement in plants. One type occurs in the parenchyma and the other in the phloem. The factors responsible for movement through the parenchyma probably are diffusion and protoplasmic streaming, perhaps aided by virus multiplication. Movement resulting from the operation of these factors would necessarily be relatively slow and independent of other materials being transported.

The factors responsible for virus movement in the phloem are not well understood, but it is quite evident that under favorable conditions viruses may move through the phloem at very rapid rates. These rates greatly exceed any movements that have been shown to occur in parenchyma.

When the respective tissue relations of the viruses of tobacco mosaic and curly top are considered in relation to the two types of movement that occur in plants, the probable reasons for the observed differences in the movement of the two viruses are more clearly evident.

Since the virus of mosaic is able to move and increase in the parenchyma, it would be expected to pass rings that destroy phloem continuity in the stems of Turkish tobacco. Matsumoto and Somazawa (13) showed that the virus occurs in the woody cylinder of tobacco stems. Since there is good evidence that it does not occur ordinarily in nonliving tissue, its presence in the woody cylinder would indicate occurrence in the wood parenchyma and in the medullary rays of the woody portions of the stem. Therefore, the virus should be able to pass rings in stems by traveling radially through medullary rays or vertically through wood parenchyma. But since travel through cells of these tissues would be slow, considerable delay in passage of rings that break phloem continuity would be expected. The results of virus passage through rings in Turkish tobacco show this expected delay.

The same principles may be used to explain the movement of a virus, such as that of tobacco mosaic, in directions opposite food flow. Comparison of the movement of the virus of curly top (4) with that of

tobacco mosaic through a horizontal stem of Turkish tobacco acropetally from an inoculated basal sucker shows that the curly top virus had in most instances either reached the top in the time interval allowed or had not moved a distance of one-half inch from the base of an inoculated sucker; whereas in the same type of plant there was a certain amount of evidence of a slow acropetal movement of the mosaic virus throughout the interval allowed for movement. If the virus of curly top is limited to the phloem it would be able to move in the phloem only when conditions were favorable for movement. Evidence indicates that conditions are not usually favorable for movement in phloem in an acropetal direction in the type of plants tested; but if they do become favorable, movement may be very rapid.

However, since the virus of mosaic is able to move in the parenchyma and since this type of movement is independent of movement in the phloem, the virus of mosaic would be able to move acropetally throughout the time allowed for movement at the rate it is able to maintain in parenchymatous cells. Although this rate of movement is not great, it would be detectable in plants held for long periods and might be appreciably greater than that in the phloem under conditions unfavorable for movement in the phloem.

The question naturally arises as to why the tobacco mosaic virus did not in all instances pass certain types of rings in *Nicotiana glauca* in which passage would have involved travel through either the medullary rays or the wood parenchyma. Not enough information is available at this time to answer this question with certainty, but the lack of mottling in infected plants may have some significance in this connection. It is conceivable that the absence of mottling in *N. glauca* was due to a low concentration or absence of virus in the chlorophyll-bearing cells. If this is true, it seems probable that much of the extraphloem tissue of the stem may be unfavorable for multiplication or movement and that the virus may be more or less closely restricted to the phloem, at least in the stem of certain individual plants. It might for this reason be incapable of traveling longitudinally through the wood parenchyma or radially through the medullary rays connecting the regions of internal and external phloem.

This concept is strengthened somewhat by the fact that the virus of cucumber mosaic, which is capable of causing distinct mottling in *Nicotiana glauca* and therefore presumably occurs in relatively high concentration in parenchyma, was able to pass rings in stems of *N. glauca* which destroyed phloem continuity and through which the virus of tobacco mosaic failed to pass.

The evidence obtained with tobacco mosaic virus in the two species of tobacco indicates that the tissue relationships of a virus may be quite different in two distinct host plants.

Similarities in the movement of the virus of tobacco mosaic and the virus of curly top are obvious when movement through the phloem is considered.

According to tests in which plants were inoculated at the top and then segmented after certain intervals, the maximum rate of movement through the phloem determined for the virus of mosaic was 36 inches in 72 hours. Evidence indicates consistently that this rate, of about one-half inch per hour, is maintained in plants under favorable conditions for movement. The virus of curly top apparently moves

at approximately the same rate in the stems of tobacco under similar conditions. Its movement in beet, however, is much more rapid.

It is recognized that there is an element of error in the determination of rates of movement of these viruses in tobacco because the time that movement begins in the phloem cannot be determined accurately. The actual maximum movement, therefore, is somewhat greater than that indicated, but probably less than twice as great. Since the element of error is present in the determination of the movement of each virus, and since its magnitude is not definitely known in either instance, accurate comparisons of the maximum movements in the stem of tobacco are not practicable. However, the evidence clearly indicates that the movement of the two viruses through the phloem of the stem of tobacco is approximately of the same order. It is evident also that both viruses move rapidly in directions of food transport and very slowly in directions opposite food transport.

The way in which the virus of tobacco mosaic moves in defoliated stems, in shoots placed in the dark, and in plants during the period of seed development, indicates clearly that movement of this virus through the stems of plants of Turkish tobacco and of *Nicotiana glauca* can be influenced, and to a considerable extent controlled, by controlling certain factors that determine the direction of movement of elaborated food materials. This seems to furnish very strong evidence in support of the view that virus movement is correlated with the translocation of elaborated food materials in the plants in which tests were made.

In view of this fact it may be well to consider further the evidence that has been interpreted as opposed to such a correlation. This evidence consists principally of the following observations. The virus moves acropetally and basipetally in the stems of normal upright tobacco and tomato plants (5, 7, 9, 10). It apparently does not move into darkened mature tobacco leaves more rapidly than it moves into normal leaves in the light, and it moves out of darkened mature leaves almost as readily as it moves out of normal leaves in the light (5). It moves out of young leaves before they are mature (6).

To interpret this evidence as opposed to the concept of correlated virus movement and food translocation, obviously the following assumptions must be made: (1) Food movement in at least the lower portions of normal upright tobacco and tomato plants is always in the direction of the roots; (2) movement of food materials is into and not out of mature tobacco leaves in the dark; and (3) no food moves out of immature tobacco leaves. It seems very doubtful that these assumptions are justified by the evidence that is available.

Since Samuel (17) found that in the stems of tomato plants the virus of tobacco mosaic usually moved first to the roots and later to the tops and Caldwell (5) found that in tobacco the virus moved first to the tops, it seems probable that movement in the stem may be in either direction, depending on conditions at the time of entrance.

It is possible that frequent directional reversals of food movement in tobacco and tomato stems may occur under normal conditions of growth and that the observed movements of virus may be correlated with diurnal or other directional reversals of food movement. The slow acropetal movement of the virus of tobacco mosaic in plants of Turkish tobacco and *Nicotiana glauca* manipulated in such a way as

presumably to decrease greatly the acropetal movement of food materials lends support to this idea.

Failure of virus to move into darkened noninoculated mature leaves of tobacco and failure of inoculated darkened leaves to restrict outward movement of virus may have an explanation that does not conflict with the concept of a correlation with food movement. It has been observed repeatedly that when mature leaves of tobacco plants are placed in the dark they soon turn yellow and die. It seems probable that such leaves do not utilize food from parts in the light, for if they did they would continue to live. Owing first to the outward movement of food reserves and later to the outward movement of products resulting from protoplasmic disintegration, the directional movements of materials in the phloem of darkened leaves of tobacco may be similar to those of normal leaves. If this is true, the directional virus movements that have been observed are those that would be expected. Results from experiments in which entire shoots were placed in the dark and in which subsequent growth showed that materials were moved into darkened shoots from parts in the light prove not only that virus can be caused to enter parts by keeping them in the dark but also that virus moves out of such parts with considerable difficulty.

No information is available regarding the stage of development that a tobacco leaf must attain before it becomes a source of supply of carbohydrates to the rest of the plant, but it seems reasonable to suspect that under favorable conditions for photosynthesis temporary surpluses of food materials may be developed in leaves long before they are mature and that at times there may be movement of materials out of relatively young leaves. Such a movement, even if it were a very limited one, would account for the observed movements of virus out of relatively young leaves.

It seems reasonable to conclude that the movement of the virus of tobacco mosaic through the phloem network of the plant is similar to that of the virus of curly top. The transport of these two viruses through the plant is probably the result of the operation of factors responsible for the translocation of elaborated food materials. The observed movements through the plant are of the type that would be expected if the basic principles of the theory of mass flow (7, 14) of liquid materials in the phloem were operating in the translocation of organic food substances.

SUMMARY

In vegetative plants of Turkish tobacco having a main stem in a horizontal position and a basal sucker in a vertical position, basipetal movement of the virus of tobacco mosaic in the main stem was rapid and acropetal movement was slow. In similar plants maturing seeds on the main stem acropetal movement was rapid. In vegetative plants, acropetal movement was accelerated by darkness and defoliation. Basipetal movement was very slow in main stems in the dark.

In plants of *Nicotiana glauca* having top and basal grafts of Turkish tobacco separated by 3 feet of stem, virus moved from the top graft to the basal graft and produced symptoms in 6 to 9 days. In 7 of 10 plants the virus failed to move from the basal to the top graft in periods ranging from 224 to 252 days. Upward movement was relatively rapid when the tops were defoliated.

Roots of Turkish tobacco were susceptible to infection, but usually long periods were required for the virus to move from the roots into the tops and produce symptoms. Removal of the tops hastened the upward movement.

The virus of tobacco mosaic moved through the rings that broke phloem continuity in the stems of Turkish tobacco, but passage was delayed. In certain plants of *Nicotiana glauca*, the virus failed to pass rings that broke phloem continuity in periods some of which were more than 250 days. However, the virus of cucumber mosaic was able to pass such rings in all cases, although passage was delayed by the rings.

Since the virus of tobacco mosaic usually does not produce mottling on *Nicotiana glauca*, it is suggested that the parenchymatous tissue of this species may be unfavorable for movement and multiplication. This may account for the inability of the virus to pass rings that break phloem continuity. The fact that the virus of cucumber mosaic, which causes mottling on *N. glauca* and therefore probably occurs abundantly in parenchyma, passed such rings in all instances lends support to this concept.

The evidence indicates that the movement of the virus of tobacco mosaic is correlated with food transport. The factors involved in movement are probably not fundamentally different from those responsible for the movement of other plant viruses.

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ORGANIC RESERVES IN THE ROOTS OF BINDWEED¹

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INTRODUCTION

Organic reserves in the roots of perennial weeds are closely associated with their persistence. Earlier work on bindweed control involved mainly the study of the effect of herbicides and the development of control methods by cultivation or smother crops with little consideration of the physiology of the weed. In 1936 work was undertaken at this station to study the carbohydrate reserves in bindweed roots and their role in growth and regeneration. The experiments reported in this paper relate to the effect of various cultural practices and chemical treatments on the trend of organic reserves in the roots of bindweed (*Convolvulus arvensis* L.).

MATERIALS

The study of the root reserves of bindweed is one phase of a larger program of weed investigations being carried on at the Colorado Agricultural Experiment Station. A 10-acre field of bindweed-infested land is under lease, and various cultural and chemical methods of weed control are in progress. The general plan of this part of the study involved the cultivation of plots at various intervals after emergence of the bindweed following each cultivation. Cultivations during the season of 1936 were (1) at emergence, (2) 3, (3) 6, (4) 9, (5) 12, and (6) 15 days after emergence. In addition to the clean cultivation and chemical tests, various cropping practices are being carried out.³

The study of the root reserves consisted essentially in determining the trend of the carbohydrates in the roots of plants under various treatments in comparison with undisturbed plants. Four separate types of collections were made:

1. Root samples collected at biweekly intervals from April 25 to October 30. Two series of plots were sampled—one in which the plants were undisturbed throughout the season, and the other in which the plants were clean-cultivated all season at 9-day intervals after emergence of the bindweed. First emergence of plants commonly occurred about 6 days after cultivating. Therefore cultivating 9 days after emergence is comparable to cultivation once every 2 weeks.

2. Root samples collected at 4-day intervals for a period of 24 days after a single cultivation at various dates during the season.

3. Root samples collected from plots treated on various dates with dry sodium chlorate at the rate of 3 pounds per square rod. Root

¹ Received for publication July 12, 1939. Contribution from the Colorado Agricultural Experiment Station in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

² Aid by Bruce J. Thornton, associate in botany, is acknowledged in supplying some of the material for table 6. He also made possible the field work incident to the preparation of this paper. Thanks are also due Dr. L. W. Durrell, head of the Department of Botany, for helpful advice and criticism in preparation of the manuscript.

³ Under the supervision of Bruce J. Thornton.

samples were taken June 1, July 1, August 1, and September 1 at time of treatment, and also 1 month after treatment. Checks were run on untreated plots.

4. Root samples were collected at the end of the growing season near October 1 from plots as follows:

(1) Uncultivated; (2) cultivated at emergence to depth of 3 inches, April to October; (3) cultivated 3 days after emergence, April to October; (4) cultivated 6 days after emergence, April to October; (5) cultivated 9 days after emergence, April to October; (6) cultivated 12 days after emergence, April to October; (7) cultivated 15 days after emergence, April to October; and (8) cultivated to a depth of 6 inches 9 days after emergence, April to October.

METHOD OF SAMPLING

Root samples were obtained by sifting the soil containing bindweed roots and placing the roots with some soil, in quart mason fruit jars for transport to the laboratory. A post-hole digger which made a hole 6 inches in diameter was used in digging the roots. By making four adjacent holes in the pattern of a square a hole was produced 1 foot square and as deep as required. For the work on bindweed, root samples were taken from the first foot level only. Five cubic feet of soil from five different locations in the plot were taken as a standard composite field sample.

The roots were taken to the laboratory as soon as possible after digging, the soil and sand removed, and the roots washed with cold water. The excess water was removed by blotting with cheesecloth. Duplicate 50-gm. samples were weighed to ± 0.05 gm. on a torsion balance. These were cut into pint mason fruit jars containing 200 ml. of boiling 95-percent alcohol, sealed, and allowed to simmer in a boiling water bath for 45 to 60 minutes. The samples were then stored until they could be conveniently extracted and analyzed.

METHODS OF ANALYSIS

The preserved root material was extracted by decantation with 80-percent boiling alcohol until reducing substances had been removed from the sample as determined by tests. From 12 to 15 extractions were required. The extract from each sample was collected in a volumetric flask and the extracting liquor made to volume after completion of the extraction. Two-hundred-milliliter portions of this liquor were used for the sugar determinations. The alcohol was removed by evaporation and the resulting water solution cleared with neutral lead acetate. Reducing substances were determined by the Munson-Walker (22)⁴ method for the precipitation of the cuprous oxide. A modification of the Bertrand (7) method was used for the estimation of copper. The residue, after the removal of the soluble carbohydrates, was dried to constant weight at 100°C., ground to suitable fineness, and analyzed for starch and acid-hydrolyzable materials separately.

One-gram samples of the residue were gelatinized and digested with undiluted taka-diastase until negative iodine tests for starch were obtained. The digest was then filtered, the filtrate cleared with neutral lead acetate, and the glucose equivalent after hydrolysis was determined on aliquots. The starch fraction included small amounts

⁴Figures in parentheses refer to Literature Cited, p. 412.

of other substances which might be brought into solution by the gelatinizing process and the taka-diastase digestion. Evidence for this is the fact that appreciable values for starch were obtained on samples from fallowed plots when iodine failed to give positive tests for starch. No important reserve material remained in the residue after the alcohol extraction and diastase digestion. This is shown by the uniformly low acid-hydrolyzable values.

The term "readily available carbohydrates," as used in this paper, includes the total sugars and starch only, since acid-hydrolyzable substances probably do not serve as a readily available reserve.

The acid-hydrolyzable fraction is shown in the tables, however, for the convenience of readers who desire to estimate the total carbohydrates. The reducing sugars are necessarily a part of the total sugars as determined after invertase hydrolysis. Results shown in all the tables are expressed on the basis of both fresh and dry weight. The graphs show the data on the basis of fresh weight.

PRESENTATION AND DISCUSSION OF RESULTS

Reserve food in the bindweed roots studied in these experiments occurred chiefly in the form of sucrose and starch. Although the reducing-sugar content was low, approximately 0.50 to 2.00 percent, the total sugars varied from about 2.00 to 7.00 percent. In a recent paper Bakke, Gaessler, and Loomis (4) showed that reserves in the bindweed roots of their experiment consisted largely of sucrose and a dextrinlike compound or group of compounds. They showed also that reducing sugars and true starch played less important roles.

TABLE 1. Seasonal variations in the carbohydrate content (percent) of cultivated and of undisturbed bindweed roots expressed on fresh-weight and dry-weight bases 1935

Date	Basis	Undisturbed plants—					Cultivated plants—				
		Reducing sugars	Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates	Reducing sugars	Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates
Apr. 25	Fresh weight...	0.53	2.44	1.80	3.12	4.24	-----	-----	-----	-----	-----
	Dry weight...	3.16	14.60	10.81	18.70	25.41	-----	-----	-----	-----	-----
May 9	Fresh weight...	.51	1.91	.93	3.10	2.84	-----	-----	-----	-----	-----
	Dry weight...	3.30	12.36	6.00	19.95	18.36	-----	-----	-----	-----	-----
May 25	Fresh weight...	.66	2.25	1.65	2.50	3.90	0.53	1.50	2.01	2.89	3.51
	Dry weight...	4.48	15.13	11.28	16.92	26.41	3.09	8.08	11.65	16.77	20.33
June 8	Fresh weight...	1.07	2.54	3.31	2.37	5.85	.40	1.77	1.40	3.00	3.17
	Dry weight...	7.13	17.77	22.09	15.85	39.86	2.48	11.00	8.56	18.70	19.56
June 22	Fresh weight...	.84	2.81	2.97	2.98	5.78	.26	1.19	.70	3.69	1.89
	Dry weight...	4.85	16.34	15.50	17.30	51.84	1.54	6.94	4.06	21.47	11.00
July 6	Fresh weight...	.77	2.66	4.20	3.20	6.86	.03	.82	.97	3.20	1.79
	Dry weight...	3.95	13.78	21.03	16.57	34.81	.18	5.02	5.97	19.40	10.99
July 20	Fresh weight...	1.31	3.94	4.74	3.15	8.98	.01	.70	.78	3.00	1.48
	Dry weight...	6.81	18.93	26.50	13.92	45.43	.09	4.52	5.22	20.17	9.74
Aug. 1	Fresh weight...	1.21	3.96	6.21	3.50	10.17	.02	.71	.79	3.33	1.50
	Dry weight...	5.24	17.05	26.81	15.10	43.86	.03	4.32	4.67	21.60	9.19
Aug. 17	Fresh weight...	1.25	2.79	5.58	3.48	8.37	-----	.71	1.00	2.80	1.71
	Dry weight...	5.63	12.56	25.15	15.72	37.71	-----	4.63	6.81	18.42	11.44
Aug. 31	Fresh weight...	1.56	3.97	6.93	3.25	10.80	.20	.98	.98	2.70	1.90
	Dry weight...	8.98	22.83	31.44	14.97	54.27	1.29	6.22	6.15	18.35	12.37
Sept. 16	Fresh weight...	1.82	4.46	8.28	3.72	12.74	.17	.54	.88	3.60	1.42
	Dry weight...	5.40	17.22	32.00	14.40	49.22	.94	3.00	4.90	19.95	7.90
Sept. 29	Fresh weight...	1.92	5.77	8.48	4.02	14.25	.11	.80	1.50	3.20	2.30
	Dry weight...	7.91	23.69	34.90	16.55	58.59	.06	4.79	9.03	19.45	13.82
Oct. 15	Fresh weight...	1.67	5.83	6.61	3.50	12.44	-----	.95	.85	2.90	1.80
	Dry weight...	9.26	32.22	29.22	15.50	61.44	-----	10.45	5.62	19.17	16.07
Oct. 30	Fresh weight...	1.00	7.20	5.22	2.93	12.32	-----	-----	-----	-----	-----
	Dry weight...	5.16	36.35	26.81	15.07	63.16	-----	-----	-----	-----	-----

In the experiments reported herein no attempt was made to separate the true starch from the dextrin. The values for starch reported, therefore, represent the true starch together with the dextrinlike group of compounds which Bakke, Gaessler, and Loomis discuss.

Table 1 shows the data for total and reducing sugars, starch, acid-hydrolyzable and the readily available carbohydrates for undisturbed plants and plants cultivated 9 days after emergence (biweekly) throughout the growing season of 1936. In most cases dates of sampling are at exactly 2-week intervals.

SEASONAL TREND OF CARBOHYDRATES IN BINDWEED ROOTS

The seasonal trend of the sugars is represented graphically in figure 1. The total sugar content of the undisturbed plants gradually increased from April to the last of October. The reducing sugars in the

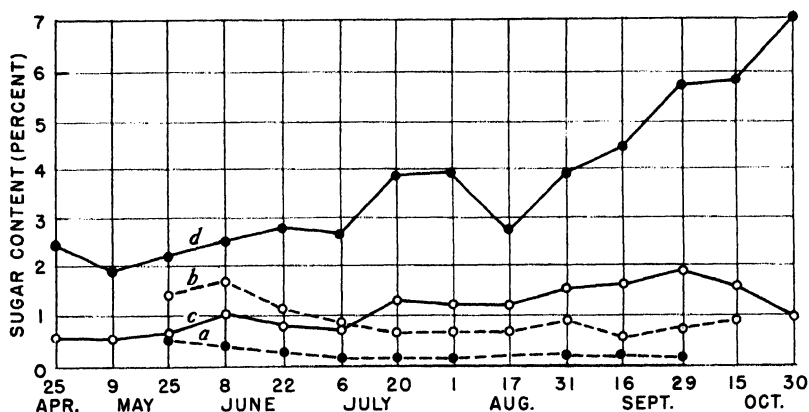


FIGURE 1.—Total and reducing sugar content of bindweed roots, when undisturbed and when cultivated at 2-week intervals, 1936: *a*, reducing sugars, cultivated; *b*, total sugars, cultivated; *c*, reducing sugars, undisturbed; *d*, total sugars, undisturbed.

undisturbed plants showed only slight increase from the beginning to the end of the experiment. Whether reducing sugars are precursors of sucrose or arise as a result of hydrolysis of the sucrose in bindweed is open to question. But in either case great variation in this fraction would not be expected, since in the former case they would rapidly be changed into the disaccharide and in the latter instance they would doubtless be used in general metabolism as rapidly as they were formed, without accumulating.

It has been shown in an earlier paper (5) that the total sugars of bindweed are materially depleted and are held at a minimum of about 1 percent of the dry weight by frequent cultivation. The data presented here show that when a cultivation program is started early in the spring the apparent depletion of total sugars is largely the result of a lack of normal accumulation rather than an actual depletion. Compare figure 1. Collection of samples from the cultivated plots was started May 25, 4 weeks after samples were first collected from the undisturbed plants. The total sugar content of the roots from the

cultivated plots was 0.75 percent less than from undisturbed plants. Slight depletion occurred, but the significant point seems to be the lack of accumulation in those plants which were repeatedly cultivated. Only traces of reducing sugars were found in the roots of cultivated plants.

In the undisturbed series, the starch content showed greater variation than did the total sugars. The minimum values for both starch and total sugars on May 9 may be accounted for by the rapid utilization of carbohydrate material for regeneration and growth in the early spring. Observation of the growth status of the plants showed that four to six leaves had appeared on the young plants before May 5; about May 7 to 9 the plants exhibited much additional growth and marked vining. It seems reasonable to believe that this rapid vegetative growth would be a drain on the reserve foods since the limited photosynthetic area probably could not supply the high sugar requirements for rapid cell division.

The data show relatively more rapid accumulation of starch than sugar before October 1. The September 29 starch value was 471 percent of the value on April 25, while the total sugar for September 29 was about 236 percent of its value on the earlier date. The marked seasonal increase in percentage of starch as compared with the accumulation of total sugars may be interpreted to mean that while sucrose may act as a temporary storage product it is rapidly transformed into the more stable and insoluble polysaccharide material.

TABLE 2.—Seasonal variations in the carbohydrate content (percent) of cultivated and of undisturbed bindweed roots expressed on fresh-weight and dry-weight bases, 1937

Date	Basis	Undisturbed plants				Cultivated plants			
		Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates	Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates
May 11.	Fresh weight.....					0.95	1.70	3.10	2.65
	Dry weight.....					5.86	13.02	21.33	18.88
May 30.	Fresh weight.....					1.59	1.74	3.14	3.33
	Dry weight.....					9.69	11.60	24.18	21.29
June 3.	Fresh weight.....	1.94	1.74	3.76	3.68	2.42	2.44	2.10	4.86
	Dry weight.....	10.16	9.96	21.20	20.12	13.90	15.75	19.70	29.65
June 17.	Fresh weight.....	1.97	2.83	3.28	4.80	2.15	3.60	3.09	5.75
	Dry weight.....	10.83	17.00	19.83	27.83	12.38	22.65	19.46	35.03
July 1.	Fresh weight.....	1.97	2.84	3.44	4.81				
	Dry weight.....	9.96	15.70	21.36	25.66				
July 15.	Fresh weight.....	1.70	3.13	3.07	4.83	1.96	4.05	2.43	6.01
	Dry weight.....	8.12	10.24	16.71	18.36	9.23	20.36	14.13	29.59
July 29.	Fresh weight.....	3.08	7.50	2.45	10.58				
	Dry weight.....	12.67	33.82	10.72	46.49				
Aug. 12.	Fresh weight.....	3.85	9.80	2.36	13.65	1.93	3.65	3.30	5.58
	Dry weight.....	16.90	41.90	10.70	58.80	9.11	17.63	14.15	26.74
Aug. 27.	Fresh weight.....	3.78	8.84	2.91	12.62	3.11	3.88	3.22	6.99
	Dry weight.....	16.70	23.90	13.40	40.60	1.27	17.69	14.70	18.96
Sept. 11.	Fresh weight.....	3.47	8.04	2.46	11.51	2.98	3.16	3.00	6.15
	Dry weight.....	13.05	35.10	10.47	48.15	14.14	16.75	15.85	30.89
Sept. 24.	Fresh weight.....	2.75	7.47	3.38	10.22				
	Dry weight.....	11.14	32.10	15.27	43.24				
Oct. 7.	Fresh weight.....	3.95	5.87	3.08	9.82				
	Dry weight.....	17.07	28.20	12.70	45.27				
Oct. 28.	Fresh weight.....	5.00	4.63	2.73	9.63				
	Dry weight.....	22.62	25.30	15.34	47.92				

The change in percentage composition of sugar and starch in the roots of bindweed after about the end of September is interesting. Field notes taken indicate that temperatures had dropped considerably and growth had practically ceased near the end of September. Superficially the plants appeared to be dead and to be going into the usual winter dormant condition. It is a well-known fact that certain plants may be hardened to make them cold and drought resistant. Chemical analysis of such plants shows a marked change in the

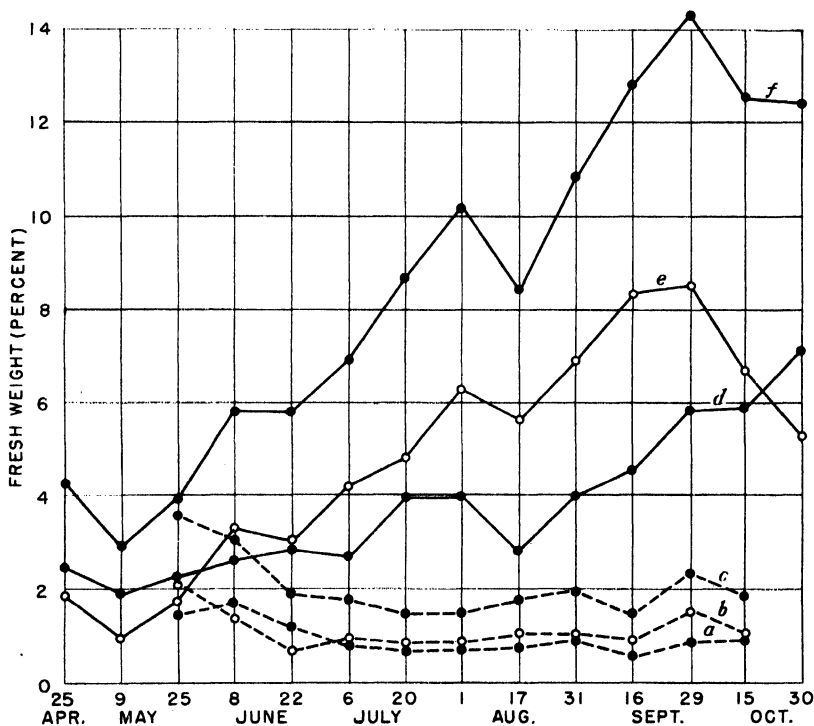


FIGURE 2.—Total sugar, starch, and readily available carbohydrate content of bindweed roots when undisturbed and when cultivated at 2-week intervals, 1936: *a*, Total sugars, cultivated; *b*, starch, cultivated; *c*, readily available carbohydrates, cultivated; *d*, total sugars, undisturbed; *e*, starch, undisturbed; *f*, readily available carbohydrates, undisturbed.

starch-sugar equilibrium. The marked decrease in starch content of bindweed roots accompanied by the increase in sugar percentage is indicative of the transition occurring in the roots with the approach of cold weather. Harvey (12) credits Lidforss (16) with reporting that a shift from polysaccharides to glucose and sucrose is a common occurrence in plants during the cold season. Hasselbring and Hawkins (13) have shown the same shift occurs in sweetpotatoes kept at low temperatures. Harvey (12) also has shown that the polysaccharides of hardened cabbages are displaced in the direction of monosaccharides and disaccharides.

Readily available reserves reached a maximum of 14.25 percent in the roots of undisturbed plants and a maximum of 3.51 percent in the cultivated plants.

Sugars, starch, and readily available carbohydrates, are plotted together in figure 2 to show their seasonal trends in undisturbed and cultivated plants. Distinct reductions are observed in each fraction. A noteworthy fact which the graph reveals is the marked reduction in the carbohydrates of the cultivated plants.

Investigation of the seasonal trend of the carbohydrates was carried through the 1937 season also. The behavior of the carbohydrate

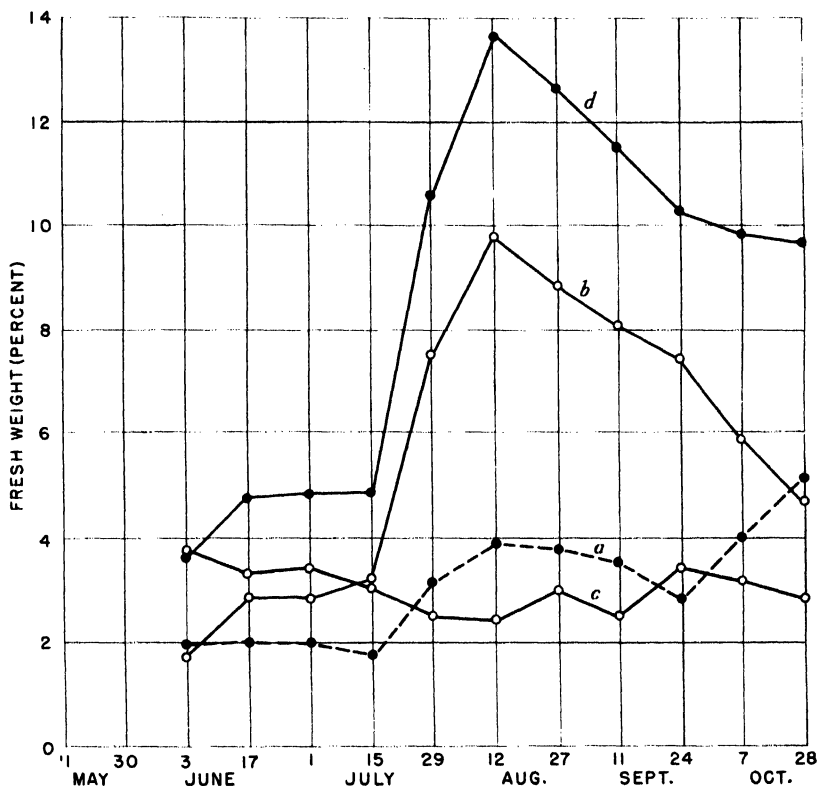


FIGURE 3.- Carbohydrate content of bindweed roots when undisturbed, 1937: a, Total sugars; b, starch; c, acid-hydrolyzable substances; d, readily available carbohydrates.

fractions in 1937 was similar to that in 1936 as shown in table 2 and figure 3. There was, however, more rapid accumulation of starch in the undisturbed plants. The data show that very little accumulation of starch occurred before July 15 but that thereafter there was a very rapid increase in the starch content, reaching a maximum of 9.80 percent by August 12th. This date was about 1 month earlier than the time of maximum carbohydrate accumulation in 1936. The data are shown in figure 4.

Weather data for 1937 are incomplete, but it is believed that the local environmental conditions delayed early accumulation and caused the rapid increase in starch between July 15 and August 12, in the undisturbed plants.

It should be pointed out that the samples used to show the seasonal trend of carbohydrates from fallowed plots for the 1937 series were taken from plots which were cultivated irregularly at 3- to 4-week intervals. This may account for the higher values found in the 1937 cultivated plants as compared with those of 1936.

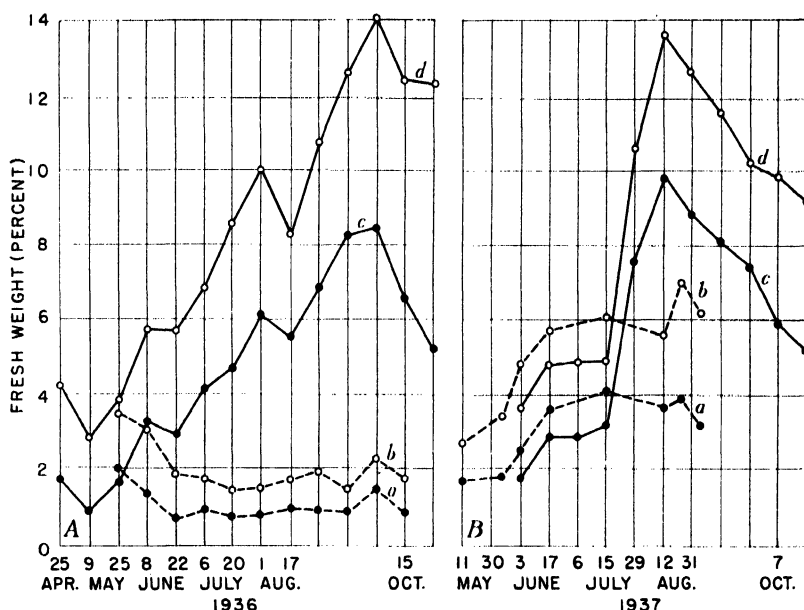


FIGURE 4.—Starch and readily available carbohydrate content of bindweed roots when undisturbed and when cultivated, 1936 (A) and 1937 (B): a, Starch, cultivated; b, readily available carbohydrates, cultivated; c, starch, undisturbed; d, readily available carbohydrates, undisturbed.

TREND OF CARBOHYDRATES IN BINDWEED ROOTS AFTER CROP HARVEST OR CULTIVATION

At the outset it was planned to study the trend of the food reserves in the roots of bindweed at short intervals of sampling after small-grain or other crop harvest and after one cultivation.

Assuming that rapid and important changes might be taking place in the carbohydrate fractions early in the season which could not be detected in the biweekly series, root samples were collected at 4-day intervals from May 25, 1936. Analyses showed, however, that reducing sugars, total sugars and acid-hydrolyzable substances remained uniformly constant. The data are given in table 3. There was a moderate decline in the starch content from May 7 to 13 which was recovered by May 17, followed by gradual accumulation until May 25. During the period May 2 to May 25 the total sugar content was about 1 percent higher than the starch content.

Root samples of bindweed were collected at 4-day intervals for a period of 24 days after barley harvest. No unusual relationship was found between the several carbohydrate fractions (table 3), as a result

of the grain crop growing with the bindweed, nor did any striking changes take place after the barley was harvested.

TABLE 3.—Variations in carbohydrate content (percent) of bindweed roots expressed on fresh-weight and dry-weight bases, 1936

[Plants sampled at 4-day intervals May 2 to 25 and July 8 to August 1 (after barley harvest)]

MAY 2 TO 25

Date	Basis	Reducing sugars	Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates
May 2	Fresh weight	0.48	1.90	1.22	3.10	3.12
	Dry weight	3.01	11.87	7.60	19.32	19.47
May 7	Fresh weight	.65	2.19	1.36	3.05	3.55
	Dry weight	4.45	13.98	8.68	19.40	22.06
May 9	Fresh weight	.51	1.91	.93	3.10	2.84
	Dry weight	3.30	12.37	6.00	19.95	18.37
May 13	Fresh weight	.65	2.15	.90	2.80	3.05
	Dry weight	4.43	14.68	6.15	19.08	20.83
May 17	Fresh weight	.69	2.37	1.39	2.97	3.76
	Dry weight	3.75	15.00	8.78	18.78	23.78
May 21	Fresh weight	.60	2.28	1.55	2.71	3.83
	Dry weight	4.24	16.15	10.96	19.17	27.11
May 25	Fresh weight	.66	2.25	1.65	2.50	3.90
	Dry weight	4.48	15.30	11.28	16.92	26.58

JULY 8 TO AUGUST 1 (AFTER BARLEY HARVEST)

July 8	Fresh weight	1.10	3.33	4.37	3.51	7.70
	Dry weight	5.80	17.63	21.03	16.87	38.66
July 12	Fresh weight	1.06	3.03	3.80	3.51	6.83
	Dry weight	5.39	15.43	19.43	17.92	34.86
July 16	Fresh weight	1.07	3.30	4.75	3.68	8.05
	Dry weight	.49	15.20	21.94	16.95	37.14
July 20	Fresh weight	1.54	4.28	4.74	3.24	9.02
	Dry weight	6.81	18.93	26.50	13.92	45.43
July 24	Fresh weight	2.32	5.14	7.71	3.49	12.85
	Dry weight	9.10	22.33	33.56	13.80	55.89
July 28	Fresh weight	2.14	5.14	7.90	3.85	13.04
	Dry weight	8.02	19.95	29.50	14.35	49.46
August 1	Fresh weight	1.23	3.96	6.22	3.50	10.18
	Dry weight	5.24	18.67	26.81	15.10	45.48

Two series of collections, usually at 4-day intervals, were made in 1937 in an effort to determine the extent of carbohydrate reduction after one cultivation. It was also believed that such a study would throw some light on the problem of carbohydrate relationships in regeneration.

In the first series the plot was cultivated May 4 and sampling was started May 11, upon emergence of the first plants. A marked decline occurred in both the sugar and starch content 4 days after emergence as compared with the values at emergence (table 4 and fig. 5). This decline was followed by a slight increase during the next 11 days. From May 11 to June 3 the starch was 0.2 to 0.8 percent higher than the total sugars. A closer relationship between these two fractions was shown here than at any other place in the study.

The first sample in the second series was collected July 29, when the plot was cultivated. Another sample was taken at emergence, and others (usually at 4-day intervals) thereafter for 24 days. Somewhat the same results were obtained at this time as earlier in the season. The total sugar content was practically the same as in the earlier series but did not show as much fluctuation. The starch content at cultivation was about 234 percent of the early spring value and dropped rapidly until 4 days after emergence and was still declining gradually at the end of the experiment.

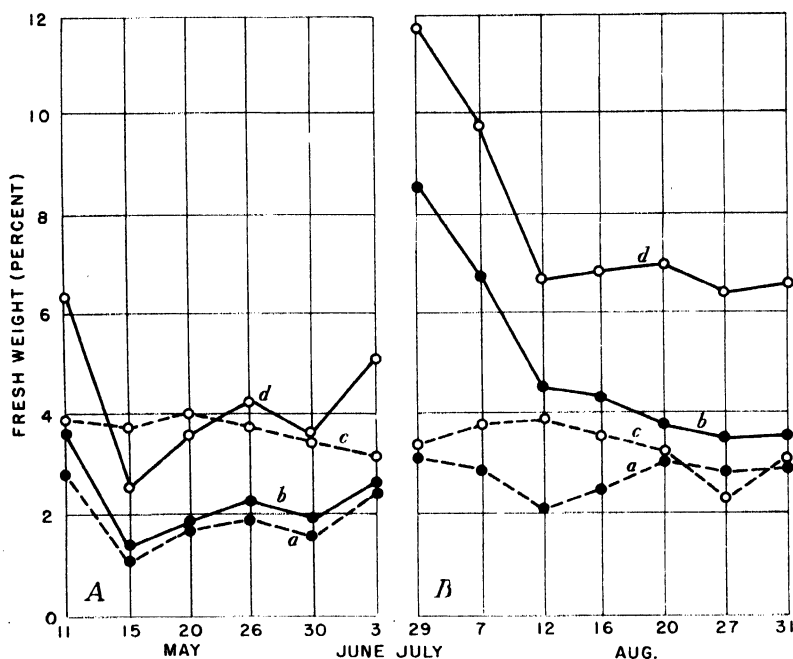


FIGURE 5.—Effect of one cultivation on the carbohydrate content of bindweed roots, 1937: *A*, Experiment performed in May; *B*, experiment performed in July. Sampled at emergence and at 4-day intervals for a period of 24 days: *a*, Total sugar; *b*, starch; *c*, acid-hydrolyzable substances; *d*, readily available carbohydrates.

TABLE 4.—Carbohydrate content (percent) of bindweed roots after one cultivation expressed on fresh-weight and dry-weight bases, 1937

MAY SERIES					
Date	Basis	Total sugar	Starch	Acid-hydrolyzable substances	Readily available carbohydrates
May 11	Fresh weight	2.78	3.61	3.93	6.39
	Dry weight	16.45	18.50	20.10	34.95
May 15	Fresh weight	1.11	1.43	3.79	2.54
	Dry weight	6.97	8.60	20.23	15.57
May 20	Fresh weight	1.72	1.92	4.06	3.64
	Dry weight	8.84	9.93	20.98	18.77
May 26	Fresh weight	1.91	2.33	3.76	4.24
	Dry weight	10.98	13.00	21.07	23.98
May 30	Fresh weight	1.59	1.91	3.43	3.50
	Dry weight	9.74	11.60	24.18	21.34
June 3	Fresh weight	2.42	2.67	3.17	5.09
	Dry weight	14.90	15.75	19.70	30.65
AUGUST SERIES					
July 29	Fresh weight	3.15	8.48	3.42	11.63
	Dry weight	12.24	33.00	13.19	45.24
Aug. 7	Fresh weight	2.95	6.75	3.84	9.70
	Dry weight	12.06	22.62	15.73	34.68
Aug. 12	Fresh weight	2.11	4.58	3.94	6.09
	Dry weight	9.13	17.63	14.15	26.76
Aug. 16	Fresh weight	2.54	4.33	3.50	6.87
	Dry weight	11.45	20.10	17.01	31.55
Aug. 20	Fresh weight	3.11	3.87	3.22	6.98
	Dry weight	1.27	17.69	14.70	18.96
Aug. 27	Fresh weight	2.87	3.52	2.34	6.39
	Dry weight	11.08	32.10	11.75	43.18
Aug. 31	Fresh weight	2.98	3.53	3.13	6.51
	Dry weight	14.14	16.75	15.85	30.89

In general, the trend of the carbohydrates after a single cultivation showed an expected decline 4 to 10 days after cultivation with only moderate change for the rest of the 24-day period. It was concluded, therefore, that 6 intervals of 4 days each was not sufficient time for the plants to recover from the set-back brought about by the cultivation, and that subsequent investigation should be performed in a somewhat different fashion.

The information gathered from the carbohydrate studies of 1935 (5), 1936, and 1937 point also to the possibility of making total carbohydrate determinations, in certain studies at least, rather than attempting to fractionate the carbohydrates.

In 1938 the study of the carbohydrate trend after one cultivation was made in the following manner: Samples were collected but instead of being preserved in alcohol, they were dried by forced ventilation in an oven at 70° C. The dry material was then ground to proper fineness and hydrolyzed with 1+20 (i. e., 1 ml. of concentrated hydrochloric acid plus 20 ml. of water) hydrochloric acid at 120° for 1 hour, and determinations were made for reducing substances.

TABLE 5. Total carbohydrates (percent) by acid hydrolysis¹ in bindweed roots after one cultivation, expressed on fresh-weight and dry-weight bases, 1938

Date	Total carbohydrates by acid hydrolysis on basis of		Date	Total carbohydrates by acid hydrolysis on basis of—	
	Fresh weight	Dry weight		Fresh weight	Dry weight
<i>June series</i>			<i>August series</i>		
June 29	5.35	33.56	Aug. 5	13.05	43.62
July 6	2.82	30.25	Aug. 26	11.20	41.10
July 11	2.99	32.82	Sept. 6	9.84	38.20
July 19	3.08	30.75	Sept. 23	10.27	38.05
Aug. 3	3.42	32.20	Oct. 1	10.96	39.98
Aug. 9	3.52	33.90	Oct. 8	11.84	42.09

¹ Analysis for total carbohydrates made by acid hydrolysis of dry tissue and determinations made by the ceric sulfate method (6).

These data are presented in table 5. In the first series shown in figure 6 A the plot was sampled June 29 at the time of cultivation, again at the time of emergence of the bindweed, and at irregular intervals for about 1 month. The second series was started August 5. Cultivation was unavoidably delayed until August 15, and samples were collected at irregular intervals after emergence until October 8. The carbohydrate content at the beginning of the second series in August (Fig. 6 B) was 244 percent of that in June. Although a reduction of 47 percent was found in the total carbohydrate content from time of cultivation until emergence in the June series, the total reduction in the August series was but 25 percent. Depletion continued longer, however, at the later date, and the subsequent accumulation of total carbohydrates was relatively more rapid.

The difference in behavior of the reserve food in the two experiments might be explained in the following manner: A certain quantity of reserve food is used for the synthesis of new protoplasts. Removal of food from a low initial reserve caused a greater net effect than later in the season when the reserve was higher. The prolonged reduction after cultivation observed later in the season was probably owing to

less favorable growing conditions. The limited growth checked the demand for reserves, and as a consequence the photosynthate accumulated rapidly in spite of the limited photosynthetic area. This accumulation then aided materially in increasing the already relatively large supply of reserve materials in the roots.

NITROGEN VARIATIONS

Studies of the organic reserves in a large number of crop plants and noxious weeds have been made by Aldous (1), Arny (2), Barr (5), Graber et al (9), Grandfield (10, 11), McCarty (19, 20), Nelson (24),

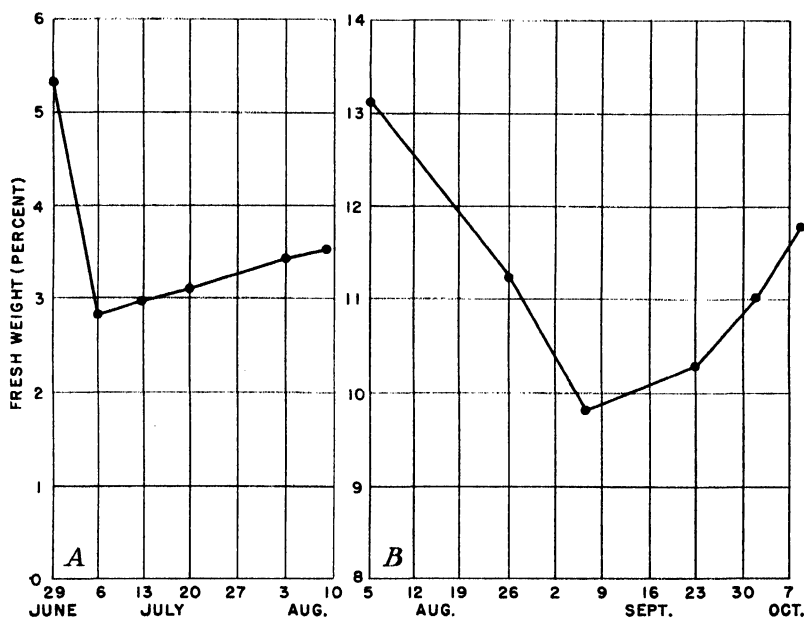


FIGURE 6.—Effect of one cultivation on the total carbohydrate content of bindweed roots, 1938: A, Experiment performed in June; B, experiment performed in August.

Pierre and Bertram (25), Welton, Morris, and Hartzler (26), and others. With few exceptions, attention has been directed chiefly to investigations of the carbohydrates. It is well known that the reserve carbohydrates are important factors in plant growth, but our knowledge relative to the several forms of nitrogen as they occur in storage organs of weeds is limited. It seemed, therefore, that a study of the various nitrogen fractions might throw some light on the problem of regeneration and growth in bindweed.

Analyses were made on the 1936 and 1937 root samples for nitrogen content. The dry material was analyzed by the Kjeldahl (3) method for colloidal or protein nitrogen. Determinations were made on the alcoholic extract for (1) ammonia and amide nitrogen according to Loomis and Shull (18), (2) amino nitrogen by the Van Slyke method, and (3) total nitrogen including nitrates by the modified Kjeldahl-salicylic acid method as described by Loomis and Shull (18). The data are given in table 6 and shown graphically in figure 7.

Table 6 and figure 7 show gradual but marked accumulation of colloidal nitrogen in the undisturbed plants from April to October. Cultivation held the colloidal nitrogen around 0.25 percent with very

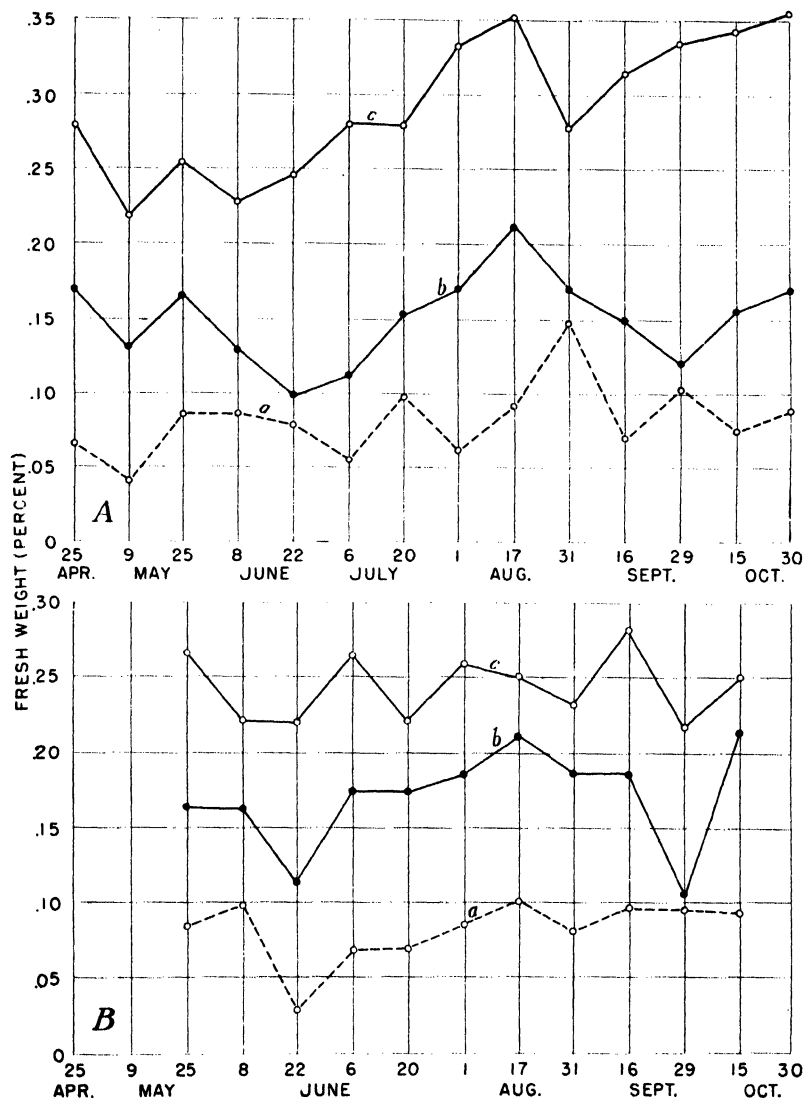


FIGURE 7.—Nitrogen content of bindweed roots when undisturbed (A) and when cultivated at 2-week intervals (B), 1936: a, Amino nitrogen; b, soluble nitrogen; c, colloidal nitrogen.

little change throughout the season. The data show that cultivation reduced the colloidal-nitrogen content, although frequency of cultivation appeared to have no material effect. No important changes occurred in the soluble-nitrogen content in either cultivated or undis-

turbed plants. Traces of amide nitrogen and moderate quantities of nitrates and nitrites which showed no consistent variations were found.

TABLE 6.—Seasonal variations in nitrogen content (percent) of bindweed roots, when undisturbed and when cultivated, expressed on fresh weight and dry weight bases, 1936-37

UNDISTURBED, 1936

Series and date	Basis	Colloidal nitrogen	Total soluble nitrogen	Amino nitrogen
Apr. 25	Fresh weight	0.27	0.17	0.067
	Dry weight	1.67	1.04	.40
May 9	Fresh weight	.21	.13	.041
	Dry weight	1.40	.84	.26
May 25	Fresh weight	.25	.16	.086
	Dry weight	1.72	1.14	.29
June 8	Fresh weight	.22	.12	.082
	Dry weight	1.51	.85	.54
June 22	Fresh weight	.24	.09	.076
	Dry weight	1.44	.79	.31
July 6	Fresh weight	.28	.11	.056
	Dry weight	1.51	.59	.29
July 20	Fresh weight	.27	.15	.098
	Dry weight	.94	.65	.43
Aug. 1	Fresh weight	.33	.17	.063
	Dry weight	1.45	.76	.13
Aug. 17	Fresh weight	.35	.21	.087
	Dry weight	1.58	.91	.39
Aug. 31	Fresh weight	.27	.17	.148
	Dry weight	1.27	.81	.68
Sept. 16	Fresh weight	.31	.15	.069
	Dry weight	1.21	.61	.31
Sept. 29	Fresh weight	.33	.12	.102
	Dry weight	1.36	.32	.42
Oct. 15	Fresh weight	.31	.15	.071
	Dry weight	1.54	.67	.31
Oct. 30	Fresh weight	.35	.17	.082
	Dry weight	1.80	.87	.45

CULTIVATED, 1936

May 25	Fresh weight	0.26	0.16	0.082
	Dry weight	1.53	.98	.48
June 8	Fresh weight	.22	.16	.098
	Dry weight	1.37	1.00	.60
June 22	Fresh weight	.21	.11	.027
	Dry weight	1.27	.65	.15
July 6	Fresh weight	.26	.17	.068
	Dry weight	1.61	1.05	.41
July 20	Fresh weight	.22	.17	.071
	Dry weight	1.48	.59	.33
Aug. 1	Fresh weight	.25	.18	.085
	Dry weight	1.56	1.10	.51
Aug. 17	Fresh weight	.24	.21	.100
	Dry weight	1.63	1.41	.65
Aug. 31	Fresh weight	.22	.18	.083
	Dry weight	1.45	1.07	.52
Sept. 16	Fresh weight	.27	.18	.095
	Dry weight	1.54	1.02	.52
Sept. 29	Fresh weight	.21	.10	.094
	Dry weight	1.39	.60	.28
Oct. 15	Fresh weight	.24	.21	.090
	Dry weight	1.64	1.38	.59

TABLE 6.—Seasonal variations in nitrogen content (percent) of bindweed roots, when undisturbed and when cultivated, expressed on fresh weight and dry weight bases, 1936–37—Continued

AFTER ONE CULTIVATION, 1937

Series and date	Basis	Colloidal nitrogen	Total soluble nitrogen	Amino nitrogen
First series:				
May 11	Fresh weight	0.25	0.26	0.089
	Dry weight	.15	1.57	.52
May 15	Fresh weight	.18	.16	.042
	Dry weight	1.16	1.07	.26
May 20	Fresh weight	.25	.25	.077
	Dry weight	1.30	1.29	.39
May 26	Fresh weight	.19	.18	.054
	Dry weight	1.14	1.07	.31
May 30	Fresh weight	.19	.15	.050
	Dry weight	1.16	.97	.30
June 3	Fresh weight	.16	.10	.053
	Dry weight	.95	.60	.31
Second series:				
July 29	Fresh weight	.27	.18	.082
	Dry weight	1.63	.68	.31
Aug. 7	Fresh weight	.22	.14	.057
	Dry weight	.91	.60	.23
Aug. 12	Fresh weight	.21	.10	.051
	Dry weight	.92	.46	.22
Aug. 16	Fresh weight	.19	.11	.046
	Dry weight	.86	.50	.10
Aug. 20	Fresh weight	.21	.14	.049
	Dry weight	.90	.57	.20
Aug. 27	Fresh weight	.20	.10	.046
	Dry weight	.89	.45	.21
Aug. 31	Fresh weight	.19	.12	.062
	Dry weight	.92	.57	.29

The most significant fluctuations were found in the roots collected at 4-day intervals after one cultivation. The soluble nitrogen content of the early-season (first) series (table 6 and fig. 8) was relatively high, having a value at the time of cultivation slightly above the colloidal nitrogen in the residue. The marked drop in both colloidal and soluble nitrogen is important and can be accounted for by the rapid utilization of nitrogenous compounds in the quick growth of the plants at that time of the year. The plot from which these samples were collected was cultivated on May 4 and showed profuse emergence on May 11 when the first sample of the series was collected. The marked fluctuation in percentage of colloidal nitrogen and total soluble nitrogen from May 11 to 20 is not understood. It seems quite probable that the initiation of new shoot growth would stimulate the digestion of stored proteins and thus reduce the percentage of these materials in the root tissue. Loomis (17) has shown that in woody plants initiation of shoot growth is correlated with a relatively high concentration of organic nitrogen compounds.

If soluble organic nitrogenous compounds are related to shoot growth in bindweed, the transition of stored proteins to soluble nitrogen would account for the rapid drop in protein after cultivation. Continued reduction of protein nitrogen for the next 10 to 15 days would be expected since considerable vegetative growth occurred during that period. This behavior is also in harmony with the trends of the sugar and starch which suggest that photosynthesis after the new plants were well established was adequate to supply the carbohydrate needs for regeneration but not sufficient to allow for any accumulation.

THE EFFECT OF SODIUM CHLORATE ON THE ORGANIC RESERVES IN BINDWEED

Observations made by a number of investigators (8, 14, 15, 21, 23, 27) have shown that sodium chlorate is generally more effective as a weed killer when applied late in the summer and fall than at any time earlier in the growing season. Unpublished results of experiments at this station with whiteweed suggested the possibility that the effect of sodium chlorate could be increased by making applications subsequent to cultivation. It was assumed that the stimulation of physiological activities by cultivation would have an initial effect of reducing the reserve food. If, then, sodium chlorate were applied, the net result

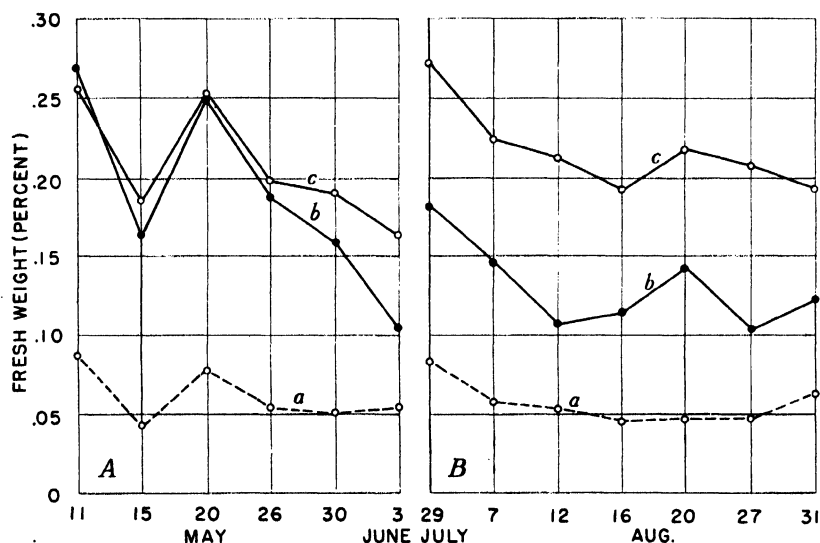


FIGURE 8.—Effect of one cultivation on the nitrogen content of bindweed roots 1937: A, Experiment performed in May; B, experiment performed in July: a Amino nitrogen; b, soluble nitrogen; c, colloidal nitrogen.

should be greater than either treatment alone. It was further believed that the application of sodium chlorate previous to cultivation probably caused the plant to become temporarily dormant which accounted for the observation that apparently better results were obtained by the addition of sodium chlorate subsequent to cultivation.

It seemed, therefore, that experiments designed to study the root reserves as affected by sodium chlorate applied to cultivated plants at various dates through the year might yield results which would be of value in developing a plant of control.

Several plots were selected which were uniformly infested with bindweed. A series of treatments was arranged so that sodium chlorate was applied to one plot June 1; another plot was treated July 1; another August 1, and so on until the middle of September. A similar series of chlorate treatments was made on plots cultivated at weekly intervals during the same period. Samples were taken when the chlorate was applied and also 1 month after its application. This arrangement provided a plan for the study of the root reserves as

affected by sodium chlorate when applied at various dates during the season and also of the effect of a combined treatment of chlorate and cultivation.

Results of analyses showed that the carbohydrate content was held to a lower value by cultivation alone than by application of sodium chlorate alone. The data of tables 7 and 8 show also that sodium

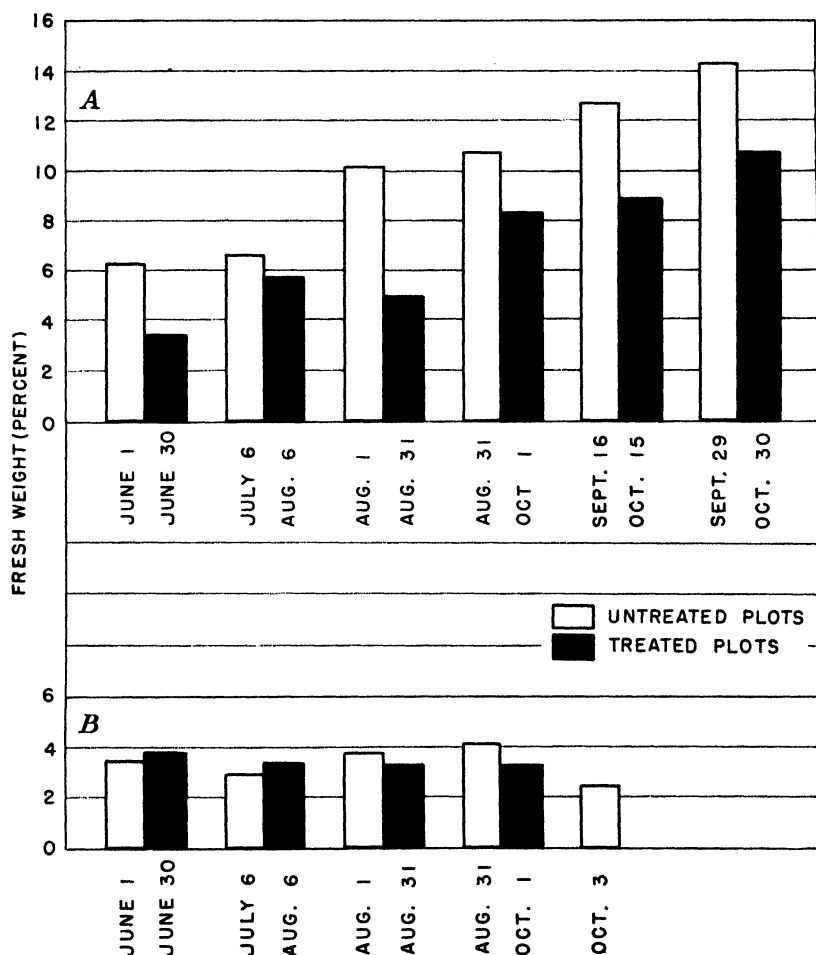


FIG. 9.—Carbohydrate content of bindweed roots as affected by date of application of sodium chlorate to undisturbed and to cultivated plots, 1936: A, Undisturbed; B, cultivated.

chlorate was relatively less effective (in reducing the carbohydrate reserve) on cultivated plants than on those which were undisturbed. The data are shown graphically in the histogram of figure 9. It may be concluded from these data that the application of sodium chlorate to bindweed under clean cultivation resulted in no important reduction of carbohydrate content beyond the decline obtained by clean cultivation.

TABLE 7.—Effect of combined treatment with sodium chlorate and cultivation on the carbohydrate content percent of bindweed roots, expressed on fresh-weight and dry-weight bases, 1936

SAMPLED AT TIME OF TREATMENT					
Date	Basis	Undisturbed plants		Cultivated plants	
		Total carbo-hydrates	Readily available carbo-hydrates	Total carbo-hydrates	Readily available carbo-hydrates
June 1	Fresh weight	9.46	6.06	6.45	3.55
	Dry weight	54.34	34.96	40.57	21.87
July 6	Fresh weight	9.76	6.56	5.27	2.87
	Dry weight	51.39	34.81	35.46	19.01
Aug. 1	Fresh weight	13.06	10.16	6.13	3.73
	Dry weight	60.58	45.48	43.52	26.22
Aug. 31	Fresh weight	13.97	10.77	7.25	4.15
	Dry weight	69.29	54.31	42.36	24.08
Sept. 16	Fresh weight	16.46	12.76		
	Dry weight	63.62	49.22		
Sept. 29	Fresh weight	18.27	14.27		
	Dry weight	75.14	58.59		
Oct. 30	Fresh weight			5.87	2.32
	Dry weight			35.22	13.92
SAMPLED 1 MONTH AFTER TREATMENT					
June 30	Fresh weight	6.52	3.32	6.44	3.71
	Dry weight	39.71	20.76	42.94	24.39
Aug. 6	Fresh weight	8.64	5.74	5.48	3.38
	Dry weight	52.02	34.17	45.31	28.06
Aug. 31	Fresh weight	8.54	4.94	5.52	3.12
	Dry weight	41.99	24.41	46.55	30.63
Oct. 1	Fresh weight	11.55	8.25	5.81	3.01
	Dry weight	55.46	39.69	38.94	20.12
Oct. 15	Fresh weight	12.52	8.82		
	Dry weight	59.01	42.06		
Oct. 30	Fresh weight	13.52	10.82		
	Dry weight	73.05	58.15		

TABLE 8.—Effect of combined treatment with sodium chlorate and cultivation on the nitrogen content (percent) of bindweed roots, expressed on fresh-weight and dry-weight bases, 1936

SAMPLED AT TIME OF TREATMENT					
Date	Basis	Undisturbed plants		Cultivated plants	
		Colloidal nitrogen	Soluble nitrogen	Colloidal nitrogen	Soluble nitrogen
June 1	Fresh weight	0.21	0.20	0.23	0.15
	Dry weight	1.24	.57	1.48	.94
July 6	Fresh weight	.28	.11	.21	.21
	Dry weight	1.51	.59	1.45	1.44
Aug. 1	Fresh weight	.33	.17	.21	.15
	Dry weight	1.45	.76	1.48	1.13
Aug. 31	Fresh weight	.22	.15	.21	.14
	Dry weight	1.26	.71	1.25	.86
Sept. 16	Fresh weight	.31	.15		
	Dry weight	1.21	.61		
Sept. 29	Fresh weight	.33	.07		
	Dry weight	1.36	.32		
Oct. 3	Fresh weight			.24	.16
	Dry weight			1.54	1.00
SAMPLED 1 MONTH AFTER TREATMENT					
June 30	Fresh weight	0.27	0.28	0.23	0.52
	Dry weight	1.61	1.65	1.56	3.74
Aug. 6	Fresh weight	.27	.16	.17	.14
	Dry weight	1.61	.96	1.35	1.19
Aug. 31	Fresh weight	.31	.16	.20	.17
	Dry weight	1.84	.82	1.31	1.12
Oct. 1	Fresh weight	.37	.20	.20	.08
	Dry weight	1.76	.97	1.34	.58
Oct. 15	Fresh weight	.39	.19		
	Dry weight	1.80	.89		
Oct. 30	Fresh weight	.33	.11		
	Dry weight	1.74	.61		

The general trend of the organic reserves in the chlorated and cultivated plants was essentially the same for 1937 as for 1936.

Estimated regrowth based on observations made in June 1939 indicated that from 50- to 75-percent eradication was obtained on uncultivated plots by the application of sodium chlorate in June, July, or August 1938 while 88- to 92-percent eradication was obtained by the September treatments. Approximately 65- to 75-percent eradication was obtained by the application of sodium chlorate in June, July, or August to cultivated plots, and 95 to 97-percent when applied in September.

TABLE 9.—*Effect of combined treatment with sodium chlorate and cultivation on the carbohydrate content (percent) of bindweed roots, expressed on fresh-weight and dry-weight, 1938.¹*

Date of cultivation and chlorate treatment		Date of sampling	Basis	Reducing sugars	Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates
Cultivated	Treated							
May 10 and June 7 None	May 10 do	June 10	Fresh weight	0.54	1.20	0.61	3.18	1.81
			Dry weight	3.83	8.40	4.31	22.41	12.71
			Fresh weight	.59	1.69	1.03	3.43	2.74
			Dry weight	3.97	11.34	7.00	22.93	18.34
May 10 and June 7 None	June 10 do	July 10	Fresh weight	1.17	2.42	.89	3.67	3.31
			Dry weight	7.27	15.03	5.56	22.80	20.59
			Fresh weight	.52	1.09	1.04	3.77	2.13
			Dry weight	3.25	6.84	6.50	23.60	13.34
May 10 and June 7 None	July 9 do	Oct 15	Fresh weight	2.23	10.53	2.01	3.46	12.54
			Dry weight	10.14	47.66	9.12	15.70	56.78
			Fresh weight	2.60	5.00	3.18	3.28	8.18
			Dry weight	11.90	27.98	14.53	14.95	42.51
May 10 and June 7 None	Aug. 5 do	Oct 22	Fresh weight	1.86	8.64	2.50	3.22	11.23
			Dry weight	7.54	40.44	12.15	15.07	52.50
			Fresh weight	2.14	5.75	1.81	2.99	7.56
			Dry weight	11.31	30.29	9.62	15.82	39.91
May 10 and June 7 None	Sept. 23 do	Oct 22	Fresh weight	.83	5.24	1.11	2.73	6.35
			Dry weight	4.18	26.43	5.59	13.75	32.02
			Fresh weight	.96	6.88	.57	1.99	7.45
			Dry weight	5.49	39.31	3.28	11.40	42.59

¹ Carbohydrates determined by use of ceric sulfate (6). Samples were taken at the time of treatment and 1 month after treatment for the first 2 months; no samples were taken following the July, August, and September treatments until the end of the season in October.

Table 9 shows the reducing sugars, total sugars, starch, and readily available carbohydrates. It should be noted that samples were taken at the time of treatments and 1 month after treatment for the first 2 months, while no samples were taken following the July, August, and September treatments until the end of the season, in October.

Results of experiments for the 3-year period 1936-38 consistently showed no important reduction of carbohydrates when attempts were made to weaken the plants by pretreatments of cultivation before applying chlorates. This is in accord with the observations made by Crafts (8) on resprouting of bindweed after hoeing and various herbicidal spray treatments.

Analysis of the dry residue for total nitrogen and the alcoholic extract for total soluble and amino nitrogen failed to give results indicating that nitrogen was significantly affected by a combined chlorate and fallow treatment.

RESERVES IN BINDWEED ROOTS AT END OF SEASON

In table 10 and figure 10 are shown reducing sugar, total sugar, starch and acid hydrolyzable values of bindweed roots collected at the end of the 1936 growing season from plots which were (1) undisturbed.

(2) cultivated all season at emergence, and (3) cultivated 3, 6, 9, 12, and 15 days after emergence. The starch content of undisturbed plants was over 700 percent of that of plants cultivated at emergence. The sugar content on the other hand in the undisturbed plants was only 360 percent of that found in the plants cultivated at emergence. Cultivation reduced the acid-hydrolyzable substances and hexose sugars only moderately, as would be expected since the acid-hydrolyzable materials do not constitute a readily available reserve and hexose sugars are present only in small quantities. Although more frequent

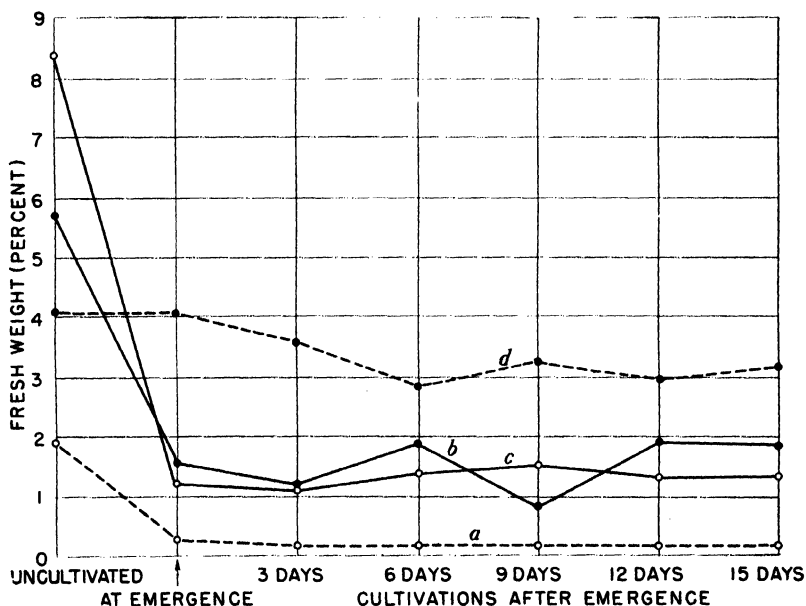


FIG. 10.—Carbohydrate content of bindweed roots when undisturbed and when cultivated at emergence and 3, 6, 9, 12, and 15 days after emergence, 1936, sampled in October: *a*, Reducing sugars; *b*, total sugars; *c*, starch; *d*, acid-hydrolyzable substances.

cultivation aided materially in diminishing the regeneration and persistence of the weed, it seemed to have no practical advantage over the less frequent cultivation (6, 9, 12, or 15 days after emergence) in reducing the values of the various carbohydrate fractions. These results are in close agreement with those reported in an earlier paper (5). The carbohydrate content of the roots of plants cultivated to a depth of 6 inches showed no important variation from that of plants cultivated to the depth of 3 inches. Data on this problem are meager, but it would seem that the advantage of deep cultivation for the purpose of reducing root reserves is open to question.

The clean-cultivation program started in 1936 was continued through 1937 and 1938. Emergence of the plants was somewhat retarded, and the time between cultivations increased for all the tests.

By October 1937 only a few scattered plants continued to emerge, and the quantity of roots which could be collected was so small that in some cases analyses could not be made. A small area of bindweed persisted, however, covering portions of two adjoining plots well into

the 1938 season after 3 years of clean cultivation at intervals of 6 and 9 days after emergence. Roots were collected, of which the analyses showed that the total sugar content was about 2.5 percent and starch about 1 percent.

TABLE 10.—Effect of cultivation on carbohydrate and nitrogen content (percent) of bindweed roots, expressed on fresh-weight and dry-weight bases, 1936

When cultivated	Basis	Reducing sugars	Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates	Colloidal nitrogen	Total soluble nitrogen	Amino nitrogen
Undisturbed	Fresh weight	1.92	5.77	8.48	4.02	14.25	0.33	0.12	0.102
	Dry weight	7.91	23.69	34.90	16.55	58.59	1.36	.32	.42
At emergence (3 inches)	Fresh weight	.27	1.59	1.19	4.02	2.78	.21	.21	.026
	Dry weight	1.56	9.26	6.90	23.35	16.16	1.27	1.26	.15
3 days after emergence	Fresh weight	.14	1.17	1.15	3.55	2.32	.24	.14	.059
	Dry weight	.86	7.02	6.90	21.30	13.92	1.49	.85	.35
6 days after emergence	Fresh weight	.20	1.89	1.36	2.81	3.25	.19	.14	.085
	Dry weight	1.60	15.23	10.90	19.40	26.13	1.52	.57	.49
9 days after emergence	Fresh weight	.11	.80	1.50	3.24	2.30	.21	.10	.094
	Dry weight	.06	4.79	9.03	19.45	13.82	1.30	.60	.28
12 days after emergence	Fresh weight	.10	1.88	1.27	2.92	3.15	.25	.11	.083
	Dry weight	1.36	25.86	7.00	16.00	32.86	1.38	.65	.45
15 days after emergence	Fresh weight	.10	1.85	1.27	3.12	3.12	.19	.10	.054
	Dry weight	.77	11.50	7.94	19.42	19.44	1.18	.67	.33
9 days after emergence (6 inches).	Fresh weight	.03	1.40	1.05	3.90	2.45	.20	.11	.073
	Dry weight	.17	8.25	5.62	20.80	13.87	1.06	.62	.38

The relatively high carbohydrate content clearly indicates movement of food from the deeper roots, since continued clean cultivation for the 2-year period had allowed no photosynthesis to take place. The results obtained here are not to be confused or, perhaps, even compared with results ordinarily obtained, since this is a single exceptional case of persistent regrowth. The behavior of the plants on this small area was very unusual and unexpected even after one season of clean cultivation. No explanation other than a possible varietal difference or peculiar soil condition can be offered to account for the persistence of the plants on the area in question.

SUMMARY

The carbohydrate and the nitrogen content of the roots of bindweed (*Convolvulus arvensis* L.) as affected by various cultural methods and chemical treatments were studied, and the results of experiments covering 3 years' investigation are reported. Data are presented to show the seasonal trend of the reducing sugars, total sugars, starch, and acid-hydrolyzable substances, for undisturbed plants and for those cultivated 9 days after emergence. The maximum reducing-sugar content did not exceed about 2 percent of the fresh weight of the roots of undisturbed plants. Total sugars reached a maximum of about 7 percent in late October, with a minimum value in May.

The percentage of starch increased gradually, reaching a maximum in August or September, followed by a sharp decline coincident with a rapid increase in percentage of total sugar. This behavior suggests the shift of polysaccharides to soluble carbohydrates which commonly occurs in plants during the cold season. Cultivation of the plants at 2-week intervals held the total sugar and starch content each to about 1 percent. The readily available carbohydrate accumulation in the roots of cultivated plants was reduced to 2.30 percent, which was 16 percent of that of undisturbed plants.

One cultivation the first part of May 1937 reduced the sugar and the starch content to about 2 percent each, while cultivation the latter part of July reduced the starch content almost 50 percent without any important effect on the total sugars. Similar experiments in 1938 showed comparable results, indicating the advantage of early fallowing.

The colloidal nitrogen in the roots of the undisturbed plants showed gradual increase from May until the last of October. Cultivation checked this accumulation. The total soluble, amino, amide, nitrate, and nitrite nitrogen fractions showed no consistent variations. On the basis of the results obtained for total nitrogen and nitrogen fraction determinations it would appear that the importance of nitrogen in a study of root reserves in bindweed is open to question.

The carbohydrate content was held to a lower value by cultivation than by application of sodium chlorate.

A combined treatment of sodium chlorate following early-season cultivation did not increase the effectiveness of the chlorate in reducing the carbohydrate reserve.

Fall applications of sodium chlorate to uncultivated plants were relatively more effective than early applications in reducing the root reserves and in controlling the weed.

Plants cultivated systematically all season at emergence, and 3, 6, 9, 12, or 15 days after emergence were uniformly low in starch content, 1 to 2 percent, as determined by taka-diastase digestion. The data showed that cultivating at emergence had essentially no greater effect than the less frequent cultivations in reducing the carbohydrate content of bindweed roots in the top foot of soil.

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DIAMETER RELATIONSHIPS OF WOOL FIBERS FROM FIVE BREEDS OF SHEEP RAISED IN SOUTH DAKOTA ¹

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INTRODUCTION

Average fiber diameter, its variability, and fiber contour are important factors in the grading of wool fiber and fleeces, inasmuch as the present wool-grading system is based on fiber diameter. While average diameter may indicate, in general, the grade of wool, the calculation of the deviation from the average is required to evince uniformity of fleece or sample. The third factor mentioned, fiber contour, has been pointed out by Barker (3, pp. 169-179) ³ as influencing the spinning qualities of wool fiber.

As part of a study of fabrics manufactured from wool from five breeds of sheep raised at the South Dakota Agricultural Experiment Station, fiber studies have been conducted on samples from three locations on each fleece. The results of these studies are reported herein. The details of sampling are given in the section on methods and materials.

REVIEW OF LITERATURE

Measurements of fiber diameter have been made by three methods: The weight-per-unit-length, the caliper, and the optical method. Krauter (10) in a paper published in 1929 presented an extensive review of the methods which had been used up to that time. Roberts (12) discussed in detail the weight-per-unit-length method which depends upon the fact that wool fiber practically always has the same specific gravity. Burns and Koehler (6) and later Burns (5) described the use of micrometer calipers for determining fiber diameter.

The optical methods which are now generally employed are of two types. The first, which was developed by the Wool Industries Research Association in England, is now recognized as official by the International Wool Textile Organization. In this case a small number of fibers are cut into very short lengths throughout the entire length of the fibers. Thus the measurement takes into account any variation in diameter that may exist along the length of the fibers. This method has been described in detail, and its applications in the study of various British breeds of sheep are reported by Wildman (17), Stanbury and Daniels (16), and Wildman and Daniels (18).

The second method, which is being widely used in the United States, is the so-called cross-section method and is recognized as an official method by the American Society for Testing Materials (2). Sidey (14)

¹ Received for publication May 18, 1930. Journal Series 113, of the South Dakota Agricultural Experiment Station, Brookings, S. Dak.

² The author wishes to express her gratitude to Dr. F. R. Immer, professor of agronomy and plant genetics, University of Minnesota, for his assistance with the statistical analyses of the data; to Barbara Webster, former student at South Dakota State College, who prepared the microscope slides; and to Dr. S. P. Swenson, associate professor of agronomy, South Dakota State College, for reviewing the manuscript.

³ Italic numbers in parentheses refer to Literature Cited, p. 425.

reported a cross-section study of New Zealand Romney and Corriedale wools in 1931. In this case the fibers were embedded in paraffin and a microtome was used for preparing the cross-section slides. Recently rapid cross-sectioning devices have been described by Hardy (1, 8) and by Schwarz (13). The cross-section procedure for diameter measurements has the advantage over the International Wool Textile Organization method in that it is possible to determine fiber contour in the same operation. This advantage becomes of particular importance in breeding work where it is desired to select animals which approach the ideal ratio of 1:1.20 (3) between the greatest and least diameters of the fibers. It is not possible, however, to record the diameter at more than a limited number of points along the length of the fiber.

An extensive study of fiber-diameter measurements was conducted by Bosman (4) on South African Merino sheep. One thousand samples were selected at random, involving 250,000 fibers. The fibers were cut into small clippings at 8 to 10 places along the fiber length and mounted, and width measurements of the longitudinal clippings were made. Eighty percent of the samples were 60's to 100's, and 11 percent were 120's to 150's.

METHODS AND MATERIALS

The procedure used to secure the fiber-diameter measurements is a modification of the cross-section method recommended by the American Society for Testing Materials (2). Samples of wool fiber were taken from the shoulder, middle, and thigh at one side of each of five different breeds of sheep—Hampshire, Rambouillet, Shropshire, Southdown, and an experimental crossbred. These sheep were selected from the flock of the department of animal husbandry of South Dakota State College. Five sheep of the Hampshire, Southdown, and crossbred breeds were sampled in 1934, six of all breeds in 1935, and nine additional crossbreds in 1938. All samples were thoroughly cleaned with carbon tetrachloride and hand-carded to mix them thoroughly. Representative samples were taken from the cleaned fiber and stained with a saturated solution of picric acid. Hardy's device (1) was used in preparing the cross-sectional microscopic slides. All samples were prepared so that the fibers were measured at the midpoint of their fiber length. The cross-section mounts were placed on a microscopic stage set at right angles to the base, and by the use of strong artificial light their images were projected onto a ground-glass plate placed at such a distance from the microscope that each fiber section was magnified approximately 600 times. Two hundred fiber sections were measured with a calibrated wedge at their greatest and least diameters at right angles to each other and to an accuracy of 1 micron as recommended by the American Society for Testing Materials (2). From these results the average diameters and the relationships between the two dimensions were calculated.

In order to draw reliable conclusions, statistical methods were employed in the analyses of the data. The mean diameter and contour ratios were determined, and the standard deviation was calculated to furnish a measure of the variability. The analysis of variance as developed by Fisher (7) was used throughout this study. The χ^2 test for homogeneity of errors was applied by using the formula suggested by Snedecor (15).

EXPERIMENTAL RESULTS

In presenting the data of the five breeds of sheep studied, it is recognized that the results obtained are not necessarily representative of these breeds as they exist universally. It is felt, however, that the data are useful in indicating the type of variation in fiber diameter and fiber contour among individual sheep, within a breed group, which may be expected when working with material of a similar type.

The usefulness of the data for this discussion becomes apparent in studies of the type for which the material was originally intended; i. e., in the study of the wool fiber used in the manufacture of experimental fabrics from fleeces of known origin. It also serves to indicate differences which may be expected in fleeces of sheep of the same breed, and in fleeces from sheep of different breeds. Descriptions of breeds and the variations to be found may be of aid to the wool grader.

In table 1 are listed the sheep of each breed studied and the average diameter and average contour of 200 wool fibers from each of three locations on each sheep; i. e., shoulder, middle, and thigh. The variability of these measurements is reported as the standard deviations of single observations.

An examination of the data in table 1 shows that there were wide variations in fiber diameter and contour between sheep within a breed, even for a given location in the fleece. The correlation coefficients for testing the association between the means and standard deviations for fiber diameters within breeds at the various positions on the fleece were calculated and tested for homogeneity according to Rider's method (11) by means of the formula:

$$\chi^2 = S(n-3)Z^2 - \frac{(S(n-3)Z)^2}{S(n-3)}$$

where

$$Z = 1/2 \log_e \frac{1+r}{1-r}$$

These correlations were found to be homogeneous, and the average correlation obtained from the weighted mean of Z was $r = +0.371$. Since Z is 4.23 times its standard error it is highly significant, and the average r (0.371) must be highly significant also.

The standard deviation of the thigh portion of the fleece was lower than that in the other portions of the fleece studied in the case of one each of the Rambouillet and Shropshire fleeces. The Rambouillet fleeces were smaller, on the average, in diameter at the middle portion than at the shoulder; and the crossbred fleeces were very slightly smaller, on the average, in the shoulder than in the middle portion. The Shropshire fleeces throughout the three portions were most uniform of the breeds studied, and the Southdown fibers were appreciably larger at the thigh than at the shoulder and middle sections.

TABLE 1.—Average diameter, average contour ratios, and standard deviations of single measurements of 200 wool fibers from each of 3 sections of fleeces from 5 breeds of sheep

Breed and section of fleece	Sample No.	Average diameter	Standard deviation ¹	Average fiber-contour ratio	Standard deviation ¹
		<i>Microns</i>	<i>Microns</i>		
Hampshire: Shoulder	1-34	24.71	5.53	1.24	0.152
	1-35	26.72	5.94	1.31	.194
	2-35	26.80	6.06	1.23	.176
	2-34	27.74	7.09	1.30	.175
	3-35	29.09	6.13	1.36	.250
	4-35	29.23	4.59	1.36	.204
	3-34	31.02	4.89	1.27	.149
	4-34	31.88	6.16	1.28	.166
	5-35	34.06	7.04	1.37	.217
	6-35	34.96	6.24	1.36	.201
	5-34	36.89	5.71	1.42	.232
Average		30.28	5.991	1.32	.1951
Middle	1-34	26.26	5.97	1.23	.144
	1-35	28.16	6.61	1.34	.215
	2-35	29.97	5.38	1.28	.194
	2-34	29.61	7.60	1.30	.202
	3-35	36.05	6.43	1.46	.313
	4-35	32.94	5.71	1.40	.197
	3-34	38.68	9.06	1.33	.191
	4-34	33.49	6.14	1.31	.186
	5-35	33.64	6.30	1.38	.263
	6-35	33.38	6.11	1.42	.273
	5-34	35.47	6.10	1.36	.233
Average		32.51	6.56	1.35	.224
Thigh	1-34	29.20	6.54	1.27	.163
	1-35	29.52	7.15	1.32	.177
	2-35	31.28	6.52	1.34	.189
	2-34	37.78	9.78	1.40	.241
	3-35	38.05	7.97	1.47	.269
	4-35	36.59	7.98	1.44	.268
	3-34	38.46	8.00	1.36	.229
	4-34	35.86	7.27	1.33	.212
	5-35	37.84	8.99	1.43	.294
	6-35	41.10	6.39	1.38	.203
	5-34	38.28	10.42	1.31	.205
Average		35.81	8.32	1.37	.226
Rambouillet: Shoulder	1-35	19.82	2.86	1.26	.135
	2-35	21.26	3.88	1.22	.165
	3-35	22.68	4.51	1.25	.137
	4-35	23.84	5.29	1.27	.151
	5-35	24.42	3.76	1.28	.195
	6-35	26.46	3.73	1.33	.202
Average		23.08	4.07	1.27	.166
Middle	1-35	21.03	3.33	1.21	.118
	2-35	20.84	3.21	1.30	.163
	3-35	21.78	3.20	1.27	.152
	4-35	21.62	5.33	1.29	.193
	5-35	22.53	4.26	1.25	.151
	6-35	23.66	3.26	1.29	.166
Average		21.91	3.85	1.27	.158
Thigh	1-35	23.58	3.47	1.25	.135
	2-35	24.13	5.78	1.25	.164
	3-35	22.24	3.98	1.33	.223
	4-35	27.04	4.93	1.32	.228
	5-35	21.24	4.44	1.25	.174
	6-35	27.58	3.99	1.38	.240
Average		24.30	4.50	1.30	.198
Shropshire: Shoulder	1-35	28.24	7.10	1.43	.241
	2-35	29.19	6.80	1.30	.173
	3-35	30.26	7.27	1.36	.202
	4-35	30.00	6.93	1.34	.203
	5-35	32.99	7.38	1.38	.216
	6-35	35.50	6.78	1.49	.299
Average		31.03	7.05	1.38	.226

¹ Average standard deviations were obtained as an average within fleeces by averaging the squared standard deviations and extracting the square root.

TABLE 1.—Average diameter, average contour ratios, and standard deviations of single measurements of 200 wool fibers from each of 3 sections of fleeces from 5 breeds of sheep—Continued

Breed and section of fleece	Sample No.	Average diameter	Standard deviation	Average fiber-contour ratio	Standard deviation
		<i>Microns</i>	<i>Microns</i>		
Middle	1-35	32.52	6.24	1.47	0.234
	2-35	29.20	6.77	1.31	.233
	3-35	29.72	7.28	1.32	.184
	4-35	33.22	7.94	1.40	.266
	5-35	36.11	7.31	1.46	.282
	6-35	34.84	7.43	1.42	.243
Average		32.60	7.18	1.40	.242
Thigh	1-35	33.20	7.78	1.48	.262
	2-35	29.14	6.35	1.32	.205
	3-35	33.38	6.62	1.45	.275
	4-35	33.75	7.66	1.36	.211
	5-35	38.42	7.27	1.47	.263
	6-35	33.98	6.63	1.39	.238
Average		33.64	7.07	1.41	.244
Southdown: Shoulder	1-34	26.66	6.17	1.31	.190
	2-34	28.48	6.94	1.30	.202
	3-34	29.22	6.56	1.18	.107
	4-34	30.32	6.46	1.27	.213
	5-34	30.67	5.18	1.28	.174
	1-35	31.05	6.35	1.26	.183
	2-35	31.05	5.31	1.26	.187
	3-35	31.87	6.73	1.26	.162
	4-35	33.63	7.22	1.33	.209
	5-35	33.94	6.63	1.28	.174
	6-35	34.11	6.32	1.27	.172
Average		31.00	6.38	1.27	.182
Middle	1-34	30.00	7.55	1.28	.194
	2-34	30.43	7.28	1.27	.170
	3-34	30.81	6.78	1.22	.136
	4-34	30.62	6.26	1.26	.213
	5-34	31.30	6.36	1.26	.161
	1-35	32.24	6.84	1.31	.232
	2-35	31.62	5.60	1.32	.153
	3-35	31.44	7.44	1.29	.174
	4-35	30.72	6.68	1.28	.170
	5-35	32.19	6.72	1.29	.204
	6-35	35.25	7.18	1.25	.167
Average		31.50	6.81	1.27	.182
Thigh	1-34	33.30	8.16	1.32	.218
	2-34	33.00	8.01	1.36	.228
	3-34	33.08	8.08	1.26	.168
	4-34	30.78	7.39	1.28	.179
	5-34	33.42	8.90	1.29	.191
	1-35	40.66	10.18	1.34	.214
	2-35	35.34	8.56	1.27	.154
	3-35	39.04	11.31	1.29	.190
	4-35	42.39	9.42	1.40	.218
	5-35	36.12	12.27	1.35	.229
	6-35	49.95	12.66	1.36	.223
Average		37.01	9.77	1.32	.203
Experimental crossbred: Shoulder	1-34	21.13	3.40	1.19	.110
	2-34	24.34	4.35	1.20	.138
	1-35	24.43	4.40	1.26	.151
	2-35	24.64	4.85	1.29	.165
	1-38	24.85	4.93	1.23	.167
	2-38	24.86	4.23	1.27	.149
	3-38	26.10	4.51	1.24	.153
	3-34	26.65	6.04	1.27	.165
	3-35	26.84	5.54	1.32	.165
	4-35	26.93	4.04	1.27	.221
	5-35	26.94	4.62	1.27	.170
	4-34	27.01	5.80	1.25	.165
	4-38	27.28	5.88	1.24	.146
	5-38	27.84	5.86	1.22	.139
	6-35	28.83	5.54	1.33	.206
	6-38	29.33	4.81	1.28	.143
	5-34	29.72	6.18	1.26	.159

TABLE 1.—Average diameter, average contour ratios, and standard deviations of single measurements of 200 wool fibers from each of 3 sections of fleeces from 5 breeds of sheep—Continued

Breed and section of fleece	Sample No.	Average diameter	Standard deviation	Average fiber-contour ratio	Standard deviation
		<i>Microns</i>	<i>Microns</i>		
Experimental crossbred Continued Shoulder	7-38	30.36	4.94	1.21	0.112
	8-38	30.52	5.28	1.23	.136
	9-38	31.82	5.66	1.29	.195
	Average	27.01	4.99	1.25	.160
Middle	1-34	23.96	4.64	1.27	.190
	2-34	26.24	4.46	1.22	.152
	1-35	26.90	4.99	1.28	.156
	2-35	24.34	4.78	1.31	.178
	1-38	24.14	4.62	1.23	.140
	2-38	26.23	5.01	1.31	.169
	3-38	27.37	5.39	1.28	.172
	3-34	27.08	7.51	1.29	.200
	3-35	29.60	7.04	1.32	.202
	4-35	27.40	4.84	1.26	.166
	5-35	27.15	4.47	1.31	.189
	4-34	26.82	7.13	1.25	.116
	4-38	29.12	4.11	1.28	.183
	5-38	26.83	5.55	1.27	.142
	6-35	24.75	5.92	1.30	.214
	6-38	28.72	5.05	1.27	.149
Thigh	5-34	27.65	5.30	1.27	.182
	7-38	28.96	5.84	1.25	.148
	8-38	28.22	4.50	1.21	.117
	9-38	31.77	5.70	1.29	.162
	Average	27.13	5.42	1.27	.170
	1-34	26.04	6.43	1.31	.219
	2-34	31.11	8.59	1.28	.193
	1-35	31.40	6.95	1.34	.230
	2-35	31.28	9.74	1.38	.258
	1-38	27.32	6.88	1.24	.141
	2-38	27.40	8.10	1.31	.197
	3-38	28.06	6.90	1.28	.154
	3-34	32.61	13.06	1.33	.253
	3-35	39.92	14.46	1.45	.281
	4-35	32.65	7.91	1.44	.270
	5-35	35.88	8.62	1.43	.265
	4-34	32.47	11.48	1.27	.195
	4-38	25.98	7.87	1.28	.189
	5-38	35.00	6.82	1.33	.203
	6-35	31.98	7.59	1.37	.255
	6-38	30.44	6.44	1.35	.192
	5-34	33.74	10.67	1.33	.233
	7-38	31.58	11.17	1.29	.163
	8-38	34.38	7.87	1.31	.204
	9-38	31.37	5.99	1.31	.190
	Average	31.53	8.98	1.33	.218

When the ratios between the two diameters of the fiber were calculated, it was found that in most instances the values for contour were appreciably higher than that given by Barker (2) for the ideal fiber. This was particularly marked in the case of the Shropshire fleeces. With the exception of the crossbred, approximately half of the fleeces showed progressively increasing contour ratios as the average fiber diameter of the three portions of the fleeces increased, while in the remaining half there was no relationship between these two factors. In the case of the crossbred fleeces, two-thirds of the fleeces exhibited increasing ratios as the diameters increased. In any one breed, however, the fleeces with the lowest diameters did not necessarily have the lowest fiber-contour ratios.

In order to determine whether the variability of the fiber diameter or contour measurements for separate locations on the fleeces of in-

dividual sheep within a breed were homogeneous, the χ^2 test for homogeneity of errors, given by Snedecor (15), was applied to the data. The results are given in table 2.

TABLE 2.—Analyses of errors of diameter and countour measurements for the different breeds of sheep by means of the χ^2 test for homogeneity

Fiber measurements and breed	Degrees of freedom	χ^2 for—		
		Shoulder	Middle	Thigh
Diameter:				
Hampshire	10	1 65.91	1 118.62	1 120.92
Rambouillet	5	1 83.16	1 122.75	1 68.77
Shropshire	5	1 2.53	1 12.01	1 14.44
Southdown	10	1 34.86	1 26.82	1 140.63
Experimental crossbred	19	1 186.93	1 269.74	1 639.99
Contour ratios:				
Hampshire	10	1 109.57	1 196.63	1 138.42
Rambouillet	5	1 61.54	1 44.66	1 83.71
Shropshire	5	1 84.53	1 35.26	1 27.48
Southdown	10	1 81.54	1 124.54	1 61.01
Experimental crossbred	19	1 249.61	1 155.75	1 249.61

¹ Exceeds the 1-percent level of significance.

² Exceeds the 5-percent level of significance.

It is seen that χ^2 , when applied to the error variances of the diameter measurements, is significant in all instances except at the shoulder in the Shropshire breed. The fleeces representing the Shropshire breed were the most homogeneous, and those of the crossbred the most heterogeneous. In the Southdown breed considerably greater variation was found in the thigh than in the shoulder and middle portions of the fleeces.

When the χ^2 test was applied to the error variances of the contour measurements of these same fleeces represented by the same fibers as were measured for diameter, the results again indicated that all of the fleeces representing each breed were heterogeneous. If all of the breeds are considered, it is noted that no one portion of the fleeces was more homogeneous than the others throughout the series. In two of the breeds the thigh sections were most homogeneous; in two others, the middle; and in the fifth breed, the shoulder sections of the fleeces.

It is apparent that variability between both fiber diameter or contour measurements of different sheep within a breed was greater than could be explained by chance, when 200 fibers were measured at a given location, except for diameter at the shoulder portion of the Shropshire breed, where only six sheep were involved in the test.

In the calculations of analyses of variance, homogeneity of the errors of the source material is assumed. As is shown in table 2, the fleeces of the five breeds studied did not exhibit this characteristic. Since, however, the data on homogeneity are the only ones which were available to describe the breed, they have been employed for the analyses of the diameter and contour measurements of the sheep within breeds. It was recognized, however, that the variability of the individual fiber measurements of different sheep was not of a homogeneous nature. In table 3 are shown the mean squares for sheep and fibers within fleeces (error) of the diameter and contour measurements. Differences between fiber measurements of the sheep within a breed were highly significant in all instances.

Table 3 shows that in the case of the mean square values for variation due to error, the values for the diameter measurements increased in progressing from the shoulder to the middle to the thigh in the Hampshire, Southdown, and crossbred breeds. The errors were lower in the middle portions of the Rambouillet, and practically the same in the three sections of the Shropshire breed. Because of the differences in mean square values for the various portions of the several breeds, it is not possible to group them and report one value as characteristic of the breed. The only instance where it would be possible to report one value for breed is in the case of the Shropshire.

The same type of relationships between the errors of the shoulder, middle, and thigh sections of the various breeds is found in the fiber-contour ratios, also shown in table 3.

TABLE 3.—Analyses of variance of the diameters and contour ratios of wool fibers from 5 breeds of sheep, variability between sheep being measured within years

Section of fleece and breed	Fiber diameter				Fiber contour ratios			
	Variation due to sheep		Variation due to error		Variation due to sheep		Variation due to error	
	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Shoulder:								
Hampshire.....	9	3,279.89	2,189	35.85	9	0.75784	2,189	0.03801
Rambouillet.....	5	1,112.00	1,194	16.59	5	.27286	1,194	.02770
Shropshire.....	5	1,437.00	1,194	49.68	5	.90560	1,194	.05105
Southdown.....	9	461.67	2,189	40.69	9	.56926	2,189	.03327
Experimental cross-bred.....	17	1,265.06	3,979	24.89	17	.18208	3,979	.02362
Middle:								
Hampshire.....	9	3,000.78	2,189	43.09	9	.64577	2,189	.05008
Rambouillet.....	5	217.40	1,194	14.80	5	.22482	1,194	.02514
Shropshire.....	5	1,503.80	1,194	51.58	5	1.14100	1,194	.05874
Southdown.....	9	298.56	2,189	46.43	9	.15821	2,189	.03300
Experimental cross-bred.....	17	708.29	3,979	20.39	17	.15758	3,979	.02872
Thigh:								
Hampshire.....	9	3,520.44	2,189	64.19	9	.64871	2,189	.05122
Rambouillet.....	5	1,295.20	1,194	20.21	5	.62344	1,194	.03909
Shropshire.....	5	1,739.20	1,194	50.04	5	.77926	1,194	.05046
Southdown.....	9	3,237.00	2,189	95.45	9	.38203	2,189	.04108
Experimental cross-bred.....	17	2,087.88	3,979	81.42	17	.32276	3,979	.04710

¹ All *F* values exceed 1-percent point.

The ratio of variation due to sheep to variation due to error (*F*) was proportionally higher for diameters than for contour. Thus, while in the contour studies, all of the values for *F* were highly significant at the 1-percent point, the results were lower than those found in the case of the diameter. The sheep varied relatively less in contour of fiber than in diameter. As was shown for the diameter studies, it is impossible to assign one value for contour as characteristic of the breed except in the case of the Shropshire.

In table 4 are given comparisons of the differences between mean fiber diameters and mean contour ratios of the different breeds for a specified location on the fleece. For such comparisons the mean squares for sheep within breeds were used to determine the significance of differences between the means of different breeds. The *t* test (?) was used for testing the significance of such differences.

TABLE 4—Mean differences¹ and their standard errors for fiber diameter and fiber contour of wool from 5 breeds of sheep clipped in 1935

Section of fleece and breed	Data for fiber diameter of breed indicated				Data for fiber contour of breed indicated			
	Rambouillet	Shropshire	Southdown	Crossbred	Rambouillet	Shropshire	Southdown	Crossbred
Shoulder:								
Hampshire.....	3 +7.06±1.74	-1.22±1.82	-2.47±1.57	3 +3.72±1.61	+0.03±0.026	-0.05±0.035	+0.05±0.024	+0.04±0.025
Rambouillet.....		3 -8.25±1.46	3 -9.53±1.13	3 -3.34±1.18		-11±.031	3 +.01±.018	3 +.02±.019
Shropshire.....			-25±1.24	3 +4.94±1.29			3 +.10±.029	3 +.09±.030
Southdown.....				3 +6.19±.90				-1.01±.016
Middle:								
Hampshire.....	3 +10.45±1.23	-24±1.61	+14±1.32	3 +5.77±1.36	+11±.029	-1.02±.040	3 +.09±.029	3 +.08±.028
Rambouillet.....		3 -5.25±1.17	-10.31±1.73	3 -4.08±.83		3 -13±.034	3 +.02±.019	3 +.03±.017
Shropshire.....			+38±1.29	3 +0.01±1.33			3 +.10±.014	3 +.10±.014
Southdown.....				3 +5.63±.96			3 +.11±.016	-1.01±.016
Thigh:								
Hampshire.....	3 +11.43±2.06	+2.09±2.17	-4.85±2.82	+1.88±2.28	3 +.10±.034	-1.01±.036	+0.7±.035	3 +.00±.028
Rambouillet.....		3 -9.34±1.38	3 -16.28±2.40	3 -8.55±1.74		3 -11±.029	3 +.03±.033	3 +.10±.029
Shropshire.....			3 -6.94±2.45	-21±1.84			3 +.08±.027	+1.01±.031
Southdown.....				3 +6.73±2.38				-1.07±.030

¹ The differences were obtained by subtracting the means of the breeds listed in the box heads from the means of the breeds in the column.3 Exceeds 1 percent, in the *t* test.3 Exceeds 5 percent, in the *t* test.

In the case of the differences between means of the diameter measurements, the differences were found to exceed the 1-percent point in the *t* test where comparisons between the Hampshire and Rambouillet and the Shropshire and Rambouillet fleeces at the shoulder, middle, and thigh; the Southdown and Rambouillet at the shoulder and thigh; the Southdown and crossbred at the shoulder and middle; the crossbred and Rambouillet at the thigh and middle; and between the crossbred and all other breeds at the middle portions of the fleeces. The Rambouillet and crossbred, and the Shropshire and crossbred were significantly different at the 5-percent point at the shoulder, and the Shropshire and crossbred and the Shropshire and Southdown at the thigh portion.

When the contour ratios were studied, the differences were significant at the 1 percent point in comparisons of the Rambouillet and Shropshire, the Shropshire and Southdown, the Shropshire and crossbred, and the Rambouillet and Southdown at the middle portion of the fleeces; and the Rambouillet and Shropshire, and the Hampshire and crossbred at the thigh. The Shropshire and Southdown and the Shropshire and crossbred showed significant differences between means at the 5-percent point at the shoulder portions; the Hampshire and Southdown, and the Hampshire and crossbred at the middle; and the Shropshire and Southdown, the Rambouillet and crossbred, and the Hampshire and Rambouillet at the thigh portion of the fleeces.

The accuracy with which the mean fiber diameter and contour of a breed can be specified depends on two components, the variability among sheep within a breed and the number of fibers measured per sheep. To demonstrate this relationship the data of the fibers from the shoulder section of the crossbred sheep are employed. The crossbred was chosen because a greater number of measurements were made for this breed and the values of the mean squares for error were relatively low. The mean error variance of the breed was 1,265.06 (table 3) divided by 4,000 or 0.3163. From these data the number of fibers necessary to measure from each fleece, if the number of fleeces were doubled, was then estimated (table 5). The formula used was one given by Immer (9).

TABLE 5.— *Estimate of number of fibers to be measured on shoulder section of crossbred breed when mean error of breed is specified*

Measurement	Mean error for breed	Sheep to be measured	Fibers required from each sheep
		<i>Number</i>	<i>Number</i>
Average fiber diameter	{ 0.3163	20	200.0
	{ .3163	40	3.9
Average fiber contour ratio	{ .0000455	20	200.0
	{ .0000455	40	25.0

The great variability between the means of the sheep compared with the comparatively much lower variability between fibers on the same sheep permits a much reduced number of fibers per sheep as the number of sheep is increased, in describing the breed fiber characteristics.

SUMMARY

The diameters of samples of wool fiber from fleeces representing five breeds of sheep were measured in cross section and their contour ratios were calculated. Wide variations in diameter between sheep within a breed, and, in most instances, between breeds were found.

Contour ratios were found, in general, to be higher than the ratio of 1:1.20 given by Barker for the ideal fiber. With the exception of the crossbred, in approximately half of the cases fiber contour was found to increase positively as fiber diameter of the various portions of a single fleece increased. Within a breed the lowest contour ratios were not necessarily found for those fleeces having the smallest diameters. In three of the breeds a majority of the fleeces showed increasing standard deviation as the contour increased. In the other two breeds, no relationships were found.

χ^2 tests for homogeneity of fleeces within a breed showed that when 200 fibers were measured per fleece it could be demonstrated that in all instances heterogeneous groups of sheep composed the samples used to represent the breeds.

In the analyses of variance for the diameter and contour measurements of sheep within breeds, *F* was found to be highly significant at the 1-percent point in all instances but one. Because of the variation in mean square values for error, it was not possible to report a single value for the breed, with the possible exception of the Shropshire breed.

Calculations of the number of fibers necessary to measure to secure the same error for the breed were made, using the shoulder measurements of the crossbred as an example. It was found that the fibers necessary would decrease rapidly if the number of sheep were increased.

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EFFECTS OF LOW TOPPING AND DIASTATIC MALT EXTRACT ON COMPOSITION AND QUALITY OF SORGO SIRUP¹

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INTRODUCTION

The present lack of uniformity in the quality of sorgo sirup may be attributed to (1) the characteristic differences in composition of juice from different varieties and from different parts of the stalk, (2) the effect of soils and fertilizers, (3) the influence of maturity and seasonal conditions, and (4) the kind of equipment, procedure, and skill employed in making the sirup. Some of these factors have previously been studied, special attention having been given to the variable composition of different parts of the sorgo stalk at several stages of maturity³ and the relationships between the content of starch, sucrose, and reducing sugar and the jellying and crystallizing characteristics of the sirups.⁴ Since the authors have reviewed the work of other investigators in earlier publications on this subject, there is no need for a detailed discussion of the literature here.

Briefly, it has been found that sirups made from the upper part of the sorgo stalk usually contain a greater percentage of starch, sucrose, mineral matter, and acidity, in proportion to dissolved solids, than do those made from the rest of the stalk. Results reported previously also show that the composition of the stalk varies considerably at different stages of maturity, the dough-to-ripe stage being considered best for sirup making. It has been found that starch is principally responsible for difficult filtration of the juice and for slow boiling, scorching, and jellying of the sirup. It has also been found that both sucrose and dextrose crystallization occur in sirups produced from different parts of the stalk of several varieties of sorgo at different stages of maturity.

DEVELOPMENT OF MALT-EXTRACT METHOD

Investigations of malt extracts and other commercial preparations containing starch-hydrolyzing enzymes were made in 1932 for the purpose of ascertaining what types of products of high starch-converting activity were available commercially at low prices. In the fall of that year, a practicable farm-scale method was developed for using malt extract of high diastatic activity on cold sorgo juice freshly expressed from the mill.⁵

¹ Received for publication Mar. 6, 1940.

² The authors gratefully acknowledge the cooperation of Martin Nelson and C. K. McClelland, of the Agronomy Department, University of Arkansas, and also that of the late J. R. Riels, director, and the late W. R. Perkins, assistant director, of the Mississippi Agricultural Experiment Station, in supplying working facilities and in connection with the agronomic work of this investigation.

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⁵ UNITED STATES BUREAU OF CHEMISTRY AND SOILS. SORGHUM SIRUP. HOW TO PREVENT JELLYING AND SLOW BOILING, AND HOW TO PREVENT SUGARING. U. S. Bur. Chem. and Soils Cir., 4 pp. 1933. [Mimeographed.]

During the season of 1934, in cooperation with the Arkansas Agricultural Experiment Station, the use of high-diastatic malt extract on sorgo juice was compared with its use on the semisirup. A severe drought during the growing season of 1934 adversely affected both the yield and the quality of all sorgo on the experimental plots. The juices from all varieties contained such a high percentage of starch that the sirups invariably scorched in the evaporator before final density was reached, except when malt-extract treatment was applied. Incidentally, on standing overnight many of the untreated sirups also clabbered or jellied. Scorching, however, rather than clabbering, was taken as the index of the efficacy of the starch-hydrolyzing treatment in the experimental work to develop the best procedure, since scorching could be noted at once while the sirups were being made. The proportion of malt extract used and the density and temperature of the semisirup to which it was added were varied until it was found under what conditions scorching could always be avoided. Similar tests were conducted on the use of malt extract in the juice. Control tests, in which no malt extract was used, were made on aliquots of the juice from all varieties. The exceptionally favorable conditions under which these experiments were carried on, particularly the uniformly high starch content of the juices, led to the development of a satisfactory method of using high-diastatic malt extract that can be depended on to yield good results, even with sorgo of extremely high starch content. The methods of using malt extract on the juice and on the semisirup have been described recently.⁶ The semisirup method is preferred to the juice method.

The objects of the present investigation were to compare the composition and quality of sirups made from the whole and from the topped stalks by a standard procedure with those of sirups made by the process in which high-diastatic malt extract was used, and to evaluate the benefits of the low-topping procedure in comparison and in conjunction with those obtained by the use of high-diastatic malt extract.

MATERIALS AND METHODS

The research was conducted in cooperation with the Mississippi Agricultural Experiment Station, eight varieties of sorgo grown by the station being utilized for this work. In general, each variety was harvested at two or three stages of maturity, and sirup was made from the whole stalks and from the topped stalks by a standard method, as a control, for comparison with sirups made by modifications of the standard procedure. In collecting the topped-stalk samples of sorgo, from two to four of the upper joints below the peduncle, depending on the length of the stalk, were discarded.

The equipment and procedure designated as standard have been described in a previous publication.⁷ In producing sirups by the standard method the juice was allowed to settle for 2 hours and was then decanted and boiled, with continuous skimming, to the density of finished sirup. The second series of samples was made by the same procedure, except that evaporation was discontinued when the density

⁶ WALTON, C. F., JR., VENTRE, E. K., and BYALL, S. FARM PRODUCTION OF SORGO SIRUP. U. S. Dept. Agr. Farmers' Bul. 1791, 40 pp., illus. 1938.

⁷ WALTON, C. F., JR., and VENTRE, E. K. EVAPORATOR FOR RESEARCH ON SIRUP MANUFACTURE. Internat. Sugar Jour. 39: 430-431. 1937.

reached 20° Baumé, measured at close to the boiling temperature, and the semisirup was allowed to settle overnight, after which it was returned to the evaporator and evaporated to final density, with the usual careful skimming. In making the third series of samples, the high-diastatic malt-extract method previously developed for treating the semisirup was used.

Duplicate samples of the finished sirup were filled when hot, direct from the evaporator, into 4-ounce screw-top bottles. Soon after the end of the sirup-making season they were analyzed for density, sucrose, invert sugar, ash, and starch. After standing for over a year, they were examined also for flavor, color, turbidity, clabbering or jellying, and crystallization.

RESULTS

The analytical data and observations regarding the quality of the sirups are given in table 1. The percentages of the components mentioned are all expressed as percentages of the weight of solids determined at sirup density by refractometer.

TABLE 1.—*Effect of topping and method of manufacture on the quality of sirups made from different varieties of sorgo at different stages of maturity*

SILVER TOP (DOUGH-TO-RIPE)

Part of stalk used for sirup	Method of making sirup	Composition of the sirup solids ¹				Observations
		Sucrose	Reducing sugars expressed as invert sugar	Ash	Starch	
		<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	
Tops.....	Standard.....	61.43	28.52	2.28	1.57	Jellied.
	do.....	52.70	42.05	2.26	.67	Very cloudy.
Whole stalk.....	Semisirup settled.....	46.80	45.97	2.30	.90	Do.
	Malt extract used on semisirup.....	49.72	45.00	2.23	.38	No jellying and settled clear.
Topped stalk.....	Standard.....	57.15	38.15	2.10	1.02	Very cloudy.
	Semisirup settled.....	57.32	39.88	1.96	.77	Do.
	Malt extract used on semisirup.....	57.28	38.12	2.14	.22	Settled clear, with no jellying.

SILVER TOP (OVERRIPE)

Whole stalk.....	Standard.....	67.46	31.75	1.90	1.00	Very cloudy.
	Semisirup settled.....	60.08	35.01	1.96	1.38	Do.
	Malt extract used on semisirup.....	58.01	37.59	1.83	.34	Settled clear, with no jellying.
Topped stalk.....	Standard.....	54.07	40.02	1.76	1.11	Very cloudy.
	Semisirup settled.....	55.15	38.61	1.85	1.24	Do.
	Malt extract used on semisirup.....	54.11	41.73	1.68	.23	Settled fairly clear, with no jellying.

RED X (DOUGH-TO-RIPE)

Tops.....	Standard.....	66.35	26.94	4.63	2.08	Jellied.
	do.....	68.28	23.16	3.15	1.92	Do.
Whole stalk.....	Semisirup settled.....	69.26	22.44	3.09	1.83	Do.
	Malt extract used on semisirup.....	66.32	25.25	2.88	.40	No jellying, and settled fairly clear.
Topped stalk.....	Standard.....	68.54	22.80	2.83	2.03	Jellied.
	Semisirup settled.....	70.00	23.93	2.84	1.49	Do.
	Malt extract used on semisirup.....	66.54	26.11	2.67	.82	No jellying, and settled fairly clear.

¹ Solids determined by refractometer.

TABLE 1.—Effect of topping and method of manufacture on the quality of sirups made from different varieties of sorgo at different stages of maturity—Continued

LEOTI RED (DOUGH-TO-RIPE)

Part of stalk used for sirup	Method of making sirup	Composition of the sirup solids				Observations
		Sucrose	Reducing sugars expressed as invert sugar	Ash	Starch	
		Percent	Percent	Percent	Percent	
Tops.....	Standard	64.75	22.30	3.91	2.84	Jellied.
	do	49.12	40.78	2.56	2.15	Do.
Whole stalk.....	Semisirup settled	44.85	49.57	2.57	1.30	Do.
	Malt extract used on semisirup.	47.20	44.00	2.31	.29	No jellying.
Topped stalk.....	Standard	42.23	48.26	2.04	1.40	Jellied.
	Malt extract used on semisirup.	47.22	44.82	1.97	.38	No jellying.

ICEBERG (RIPE)

Whole stalk.....	Standard	63.06	30.05	1.93	1.25	Much fine grain (sucrose crystallization due to extra high density).
	Semisirup settled	64.63	30.14	1.75	1.08	Cloudy.
	Malt extract used on semisirup.	64.20	32.19	1.66	.38	No jellying; no sugaring; settled clear.
Topped stalk.....	Standard	53.73	42.51	1.75	.65	Cloudy.
	Semisirup settled	53.00	42.15	1.68	.88	Do.
	Malt extract used on semisirup.	51.68	44.85	1.76	.30	No jellying; no sugaring; settled clear.

ICEBERG (OVERRIPE)

Whole stalk.....	Standard	72.30	21.46	2.22	1.46	Jellied solid, with 2 large sucrose crystals on top.
	Malt extract used on semisirup.	69.22	26.01	2.18	.33	No jellying; settled clear; rock-candy crystallization.
Topped stalk.....	Standard	62.09	30.25	2.23	1.66	Jellied solid.
	Malt extract used on semisirup.	62.80	33.73	2.15	.26	No jellying; settled clear.

KANSAS ORANGE (DOUGH-TO-RIPE)

Tops	Standard	53.28	34.16	4.28	1.28	Jellied.
	do	52.97	39.97	2.96		
Whole stalk.....	Semisirup settled	43.47	51.08	2.30	1.02	Started to jelly.
	Malt extract used on semisirup.	42.97	51.44	2.29	.36	No jellying; no sugaring; very clear.
Topped stalk.....	Standard	67.24	24.68	2.17	1.49	Jellied, with sucrose crystallization.
	Malt extract used on semisirup.	65.23	28.64	2.10	.58	No jellying; very clear; sucrose crystallization.

JAPANESE SEEDED RIBBON (RIPE)

Whole stalk.....	Standard	28.46	66.69	2.09	0.56	Cloudy.
	Semisirup settled	25.66	68.57	2.08	.53	Do.
	Malt extract used on semisirup.	23.98	69.60	2.08	.23	Clear.
Topped stalk.....	Standard	15.31	79.22	1.86	.30	Cloudy.
	Semisirup settled	18.65	77.98	1.98	.35	Do.
	Malt extract used on semisirup.	17.84	78.42	1.91	.24	Clear and dextrose crystallization.

TABLE 1.—*Effect of topping and method of manufacture on the quality of sirups made from different varieties of sorgo at different stages of maturity—Continued*

JAPANESE SEEDED RIBBON (DEAD RIPE)

Part of stalk used for sirup	Method of making sirup	Composition of the sirup solids				Observations
		Sucrose	Reducing sugars expressed as invert sugar	Ash	Starch	
Tops	Standard	Percent 43.69	Percent 50.00	Percent 3.50	Percent 1.02	Opaque and greenish.
Whole stalk	do	39.81	55.49	1.86	1.02	Cloudy.
	Malt extract used on semisirup	40.76	53.62	1.84	.32	Settled clear.
Topped stalk	Standard	24.80	64.05	1.55	.72	Cloudy.
	Semisirup settled	25.16	71.51	1.64	.45	Do.
	Malt extract used on semisirup	20.31	75.34	1.59	.27	Clear.

GOOSENECK (RIPE)

Whole stalk ..	Standard	51.03	41.32	2.25	1.01	Opaque and very viscous.
	Semisirup settled	51.76	40.73	2.16	1.03	Do.
	Malt extract used on semisirup	51.96	42.34	1.95	.34	Settled clear and low viscosity.
Topped stalk ..	Standard	40.99	52.01	2.04	.62	Opaque and viscous.
	Malt extract used on semisirup	42.69	52.58	1.73	.13	Settled clear and low viscosity.

GOOSENECK (OVERRIPE)

Whole stalk ..	Standard	57.95	34.01	2.46	1.22	Jellied.
	Malt extract used on semisirup	59.17	35.68	2.22	.37	Settled clear and low viscosity.
Topped stalk ..	Standard	49.48	43.88	2.30	.97	Opaque and viscous.
	Malt extract used on semisirup	47.57	46.03	2.04	.14	Settled clear and low viscosity.

HODO (GREEN)

Whole stalk ..	Standard	26.25	69.18	2.53	(1)	Slightly cloudy.
	Semisirup settled	27.18	67.35	2.49	(1)	Do.
	Malt extract used on semisirup	25.84	69.03	2.49	(1)	Clear.
Topped stalk ..	Standard	39.35	64.53	2.46	(1)	Slightly cloudy.
	Semisirup settled	30.48	64.79	2.46	(1)	Do.
	Malt extract used on semisirup	30.02	64.40	2.40	(1)	Clear.

HODO (RIPE)

Whole stalk ..	Standard	48.23	46.42	2.43	0.25	Cloudy.
	Semisirup settled	46.60	47.98	2.24	.16	Slightly cloudy.
	Malt extract used on semisirup	46.60	47.98	2.19	(1)	Clear.
Topped stalk ..	Standard	48.23	45.82	2.38	.15	Slightly cloudy.
	Semisirup settled	47.13	46.66	2.36	.11	Do.
	Malt extract used on semisirup	47.30	46.94	2.36	(1)	Clear.

HODO (OVERRIPE)

Tops	Standard	40.20	53.21	3.47	0.34	Very cloudy.
Whole stalk ..	do	52.78	41.84	2.65	.20	Cloudy.
	Semisirup settled	49.11	45.06	2.60	.12	Do.
	Malt extract used on semisirup	49.31	44.98	2.56	(1)	Settled clear.
Topped stalk ..	Standard	46.13	47.94	2.26	.08	Cloudy.
	Semisirup settled	42.23	52.17	2.25	.08	Slightly cloudy.
	Malt extract used on semisirup	42.35	52.33	2.19	(1)	Clear.

¹ Negative.

DISCUSSION

Some of the samples exhibited sucrose crystallization when the sucrose content was approximately 65 percent, or higher, and the density was higher than 78° Brix, while one with high invert sugar content showed dextrose crystallization. As the sirup samples varied somewhat in density, however, little attention was given to crystallization, either as a varietal characteristic or as related to the composition of the sirups.

Most of the sirups with a starch content of 1.25 percent or higher jellied or became extremely viscous, whereas those made by the use of high-diastatic malt extract had a starch content considerably under 1.0 percent, in many cases only about 0.25 percent, and did not jelly.

The ash content of the samples varied considerably with the variety but it was usually higher in sirups made from the top portion of the stalk. As a rule, sirup from the tops likewise contained a greater proportion of starch.

In making sirups with juice from the tops alone, it was observed that scorching usually occurred before evaporation to the standard density of sirup was completed. This is characteristic of juices of high starch content, and accounts for the dark color and strong flavor of some sirups.

Whenever diastatic malt extract was used in proper amount, the resulting sirup possessed greatly improved clarity, in comparison with the corresponding untreated sample, and also deposited its sediment more rapidly. The relatively high starch content of some sirups doubtless acts as a stabilizer, holding other nonsugars in suspension and giving a more turbid product.

SUMMARY AND CONCLUSIONS

Sirups made by a standard procedure from the whole stalks and from the topped stalks of eight varieties of sorgo were compared with sirups made by modifications of this procedure, special attention being given to the use of starch-hydrolyzing enzymes.

The topped stalks consistently produced sirup of better quality than the whole stalks, which, in turn, gave better sirup than did the tops alone.

Relatively little improvement in quality resulted from simply allowing the semisirup to settle, without malt-extract treatment, before completing the evaporation.

All sirups produced by the process in which high-diastatic malt extract was used were of better color, flavor, and clarity than the sirups made from corresponding parts of the stalk by the standard procedure.

The results show that sirups of the highest quality are produced by using starch-hydrolyzing enzymes to supplement reasonably good topping practice.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 60

WASHINGTON, D. C., APRIL 1, 1940

No. 7

THREE PREVIOUSLY UNDESCRIBED MOSAIC DISEASES OF PEA¹

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INTRODUCTION

During the last few years a number of distinct viruses affecting pea (*Pisum sativum* L.) have been named and described. In the course of experimental studies on varietal susceptibility and resistance of peas to a virus from mosaic-infected alsike clover, the writer found three other viruses on peas which produced symptoms somewhat unlike those already described on certain varieties. Studies were then conducted to determine the symptoms produced by these viruses on peas and beans and the resistance and susceptibility of a number of varieties of each. Host range and physical properties of the viruses in vitro were also investigated. These studies disclosed sufficient differences to permit classification and identification. A comparative study of these viruses and of an earlier described mosaic virus from alsike clover likewise infectious to pea is presented in this paper.

REVIEW OF LITERATURE

Comprehensive reviews of the literature on the mosaic diseases of peas have been made by Pierce (10),³ Zaumeyer and Wade (16), Stubbs (11), and Murphy and Pierce (4). Johnson and Jones (1) in 1937 described two mosaic diseases of pea in Washington, one an enation mosaic and the other a severe mosaic. The former is identical with the pea enation virus disease described by Osborn (5), Pierce (10), and Stubbs (11), which has been designated as pea virus 1. Severe mosaic, according to Johnson and Jones (1), appears to be similar to pea virus 3. Zaumeyer and Wade (17) recorded the varietal susceptibility of a number of pea varieties to pea streak virus 1, which is unlike any previously described virus of peas. Johnson and Lefebvre (2) reported a crotalaria mosaic that was infectious to a number of species of this host as well as to peas and other legumes. It is not known whether this virus is distinct from the viruses previously reported. Whipple and Walker (13) described two strains of the cucumber mosaic virus which infected pea and bean. Zaumeyer (14) compared pea streak virus 1 with three strains of the alfalfa mosaic virus, all of which infected pea. Wade and Zaumeyer (12) recorded varietal susceptibility and resistance of a number of pea varieties to a virus from alsike clover which is identical with the one described here as alsike clover mosaic virus 1. Osborn (8) recently reported on the incubation period of pea virus 1 in the aphid *Macrosiphum solanifolii* Ash. and later (9) reported strains of this virus.

¹ Received for publication January 11, 1940.

² The writer is indebted to L. L. Harter for suggestions and criticisms in the preparation of the manuscript.

³ Italic numbers in parentheses refer to Literature Cited, p. 451.

Zaumeyer (15) compared two new viruses of the pea which resemble those reported by Whipple and Walker (13).

Pierce (10) arbitrarily classified a number of legume viruses which had been previously reported upon by him as well as by other investigators. His classification was based on the host from which the virus was collected, i. e., beans, peas, white clover, etc. Following this system of classification, the writer has designated the viruses herein discussed as pea mosaic viruses 4 and 5 and alsike clover mosaic viruses 1 and 2.

MATERIALS AND METHODS

SOURCES OF VIRUSES

Pea mosaic virus 4.—This virus was obtained from mosaic-infected peas grown in northeastern Colorado. The symptoms produced, which are described later, were in general mild on the several pea varieties tested (fig. 1, *A* and *B*).

Pea mosaic virus 5 (pea stunt mosaic).—Specimens from which this virus was isolated were collected from the Potlatch variety near Avon, Colo. This virus produced a decided stunting on the Dwarf Telephone variety (fig. 2, *A* and *F*).

Alsike clover mosaic virus 1.—This virus was obtained from a naturally infected alsike clover plant growing in northeastern Colorado. The symptoms produced on most pea varieties were mild and somewhat similar to those produced by pea mosaic virus 4 (fig. 3, *C* and *D*).

Alsike clover mosaic virus 2.⁴—This virus was secured from an alsike clover plant collected near Stonewall, Colo. It produced symptoms on alsike clover similar to those of alsike clover mosaic virus 1 but caused distinctly different symptoms on peas. Alsike clover mosaic virus 2 produced very marked symptoms on most varieties of peas (fig. 4, *A, B*, and *I*).

METHODS

The methods employed in these studies were similar to those described by other investigators who have worked with viruses of leguminous plants (4, 9, 10, 13, 17). The work was carried out entirely under greenhouse conditions at temperatures ranging from 18° to 25° C. Carborundum powder No. 38713 was dusted on the leaves before they were mechanically inoculated with the virus extracts.

The methods used in the studies of thermal inactivation and dilution were similar to those described by Johnson and Grant (3). For the aging tests, the juice of infected plants was stored in stoppered flasks and kept in a darkened chamber maintained at a constant temperature of 18° C. Juice was taken from the flasks at 24-hour intervals, and inoculations were made in the usual manner.

EXPERIMENTAL RESULTS

The viruses dealt with in this paper may be differentiated (1) by the symptoms they produce on several varieties of pea and bean; (2) by the susceptibility and resistance of certain varieties of pea,

⁴ Since the preparation of this article, a new classification of the typical legume viruses has been published: WEISS, F. A KEY TO THE TYPICAL VIRUSES OF THE LEGUMINOUS CROPS. U. S. Bur. Plant Indus., Plant Dis. Rpt. 23: 352-360. 1939. [Mimeographed.]

In this classification, pea mosaic virus 4 has been changed to *Pisum virus 3A*, pea mosaic virus 5 to *Pisum virus 5*, alsike clover mosaic virus 1 to *Trifolium virus 3*, and alsike mosaic virus 2 to *Trifolium virus 3A*.

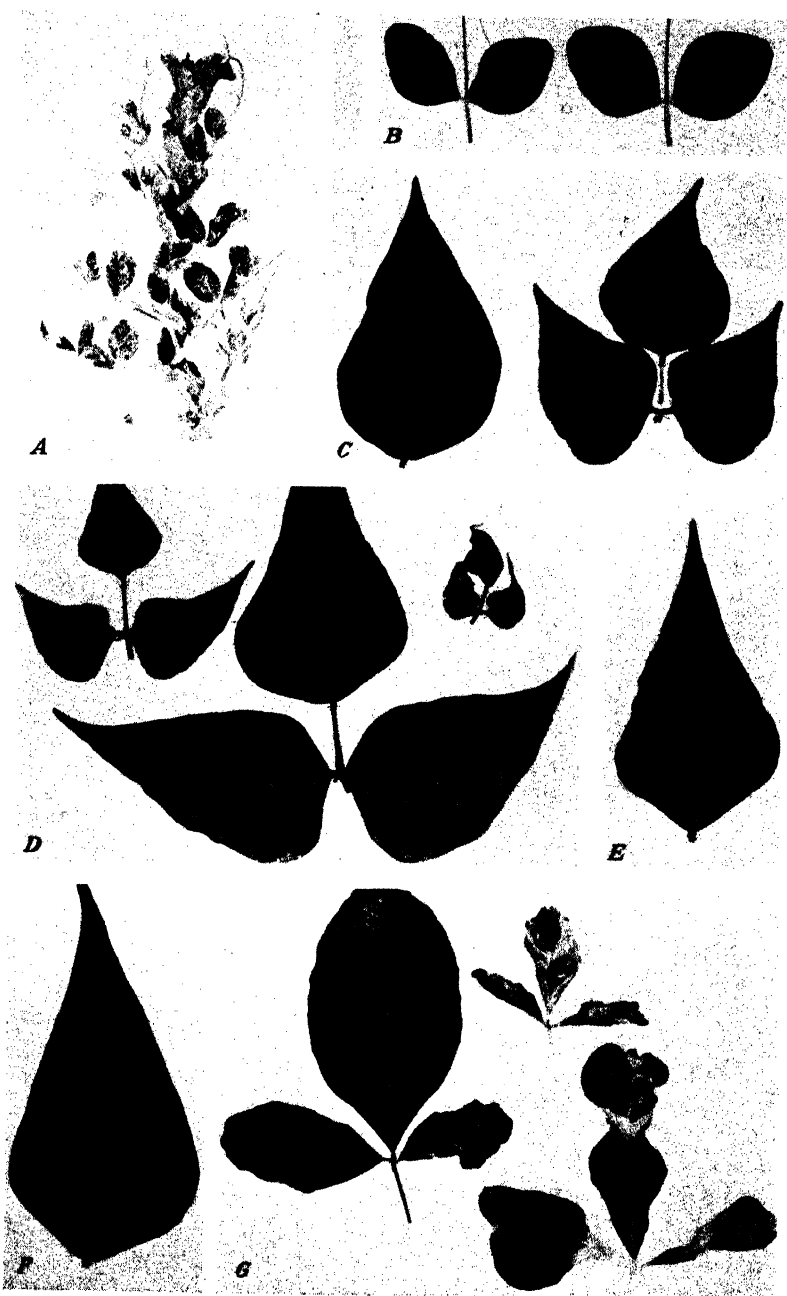


FIGURE 1.—Symptoms produced by pea mosaic virus 4 on several hosts: A, B, Leaf mottling on Green Giant pea; C, leaf mottling on Robust bean; D, leaf mottling on Stringless Green Refugee bean; E, F, halolike yellow spotting on U. S. No. 5 Refugee bean; G, leaf mottling and malformation on *Crotalaria spectabilis*.

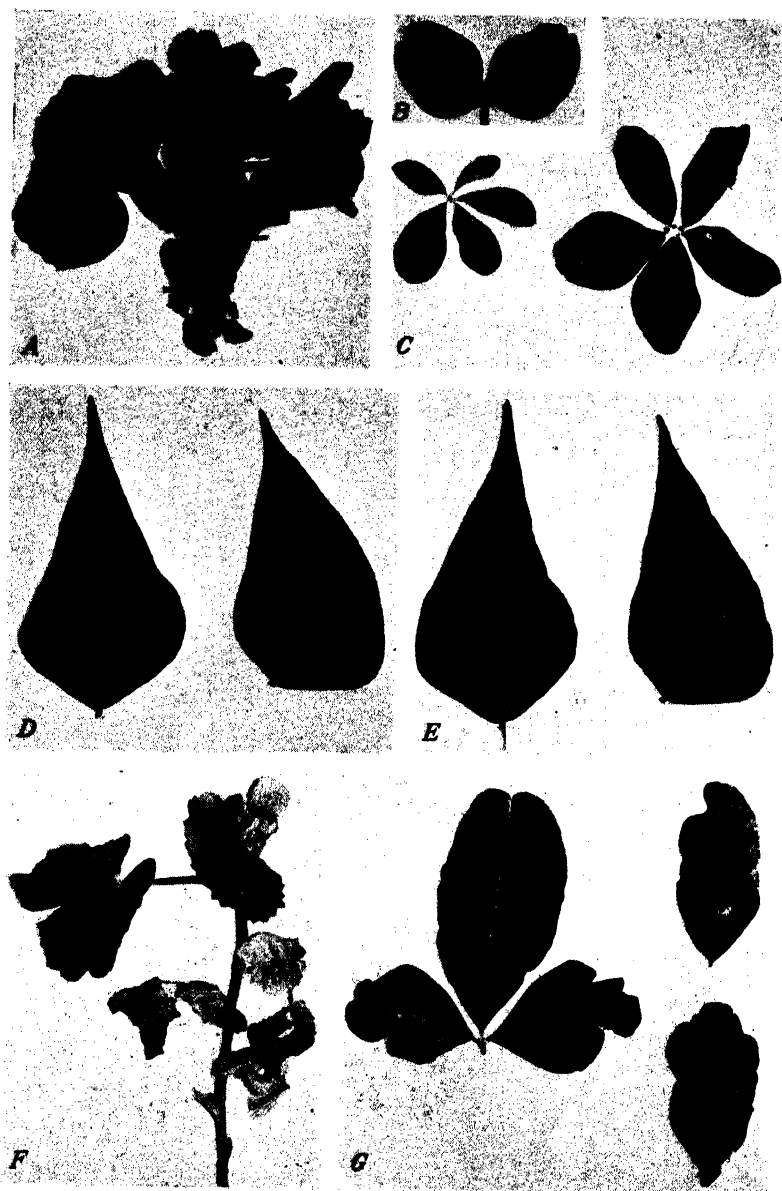


FIGURE 2.—Symptoms produced by pea mosaic virus 5 on several hosts: *A*, Stunting and terminal rosetting of Dwarf Telephone pea; *B*, leaf mottling on Green Giant pea; *C*, leaf malformation and necrosis on white lupine; *D*, mild leaf mottling on Idaho Refugee bean; *E*, leaf mottling on Stringless Green Refugee bean; *F*, leaf necrosis and terminal killing of Dwarf Telephone pea; *G*, leaf mottling and malformation on *Crotalaria spectabilis*.

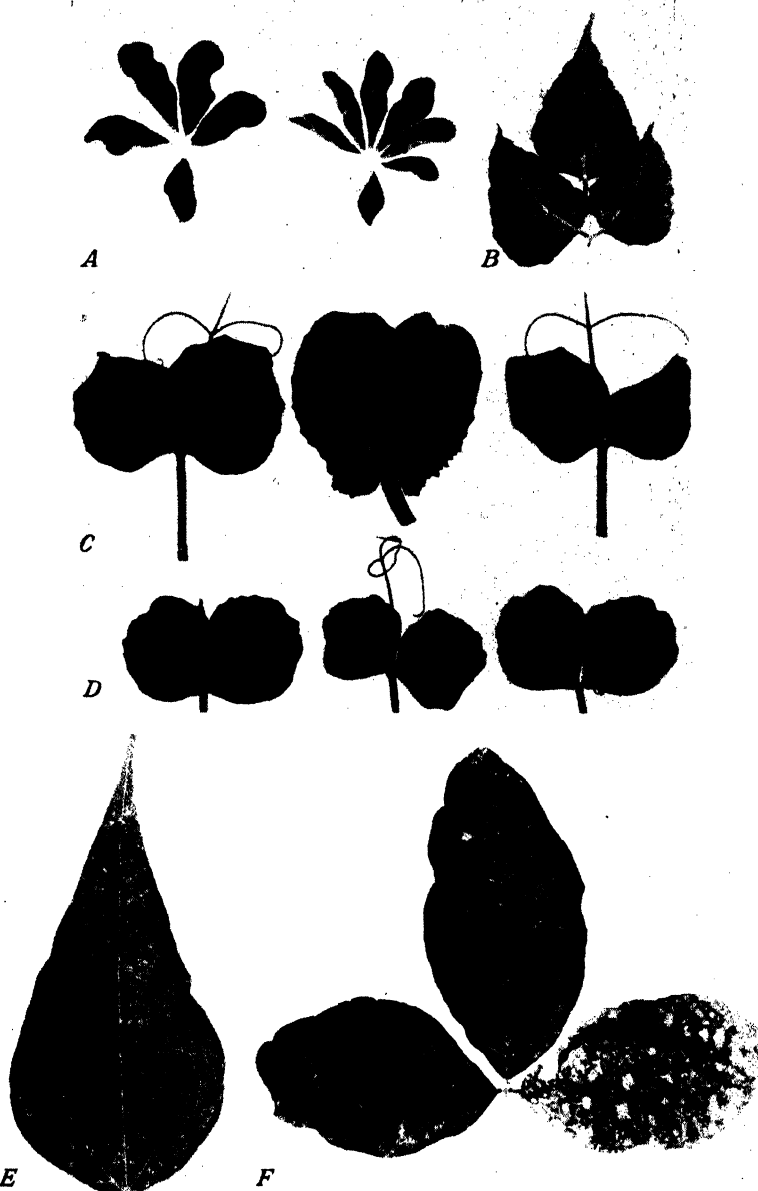


FIGURE 3.—Symptoms produced by alsike clover mosaic virus 1 on several hosts: *A*, Leaf mottling on white lupine; *B*, leaf mottling and puckering on Robust bean; *C*, *D*, leaf mottling on Dwarf Telephone and Green Giant pea, respectively; *E*, yellow leaf spotting on Stringless Green Refugee bean; *F*, yellow leaf spotting on *Crotalaria spectabilis*.



FIGURE 4.—Symptoms produced by alsike clover mosaic virus 2 on a number of hosts: *A*, Necrosis and killing of leaves of Green Giant pea; *B*, pod spotting on Alaska pea; *C*, *E*, *F*, leaf mottling and malformation on Corbett Refugee bean; *D*, leaf mottling on Robust bean; *G*, pod of healthy Alaska pea; *H*, trifoliate leaves of Idaho Refugee bean, showing yellow spotting and mottling; *I*, leaf spotting and necrosis on Dwarf Telephone pea; *J*, leaf malformation and death of white lupine; *K*, healthy Dwarf Telephone pea; *L*, healthy white lupine.

bean, and other legumes and hosts; and (3) by the properties of the viruses in vitro.

SYMPTOMS PRODUCED ON PEAS

Although symptomatology alone is not always sufficient to enable one to identify certain viruses on pea, a careful study of the symptoms produced on differential hosts is helpful. Some of the viruses here reported caused consistent symptoms on a number of pea varieties that can be relied on to help differentiate them. The descriptions of the symptoms produced by the several viruses are based on those that developed on Dwarf Telephone, Telephone, and Green Giant varieties, which were artificially inoculated in the greenhouse.

PEA MOSAIC VIRUS 4

Pea mosaic virus 4 caused rather mild symptoms on most of the pea varieties tested (fig. 1, *A* and *B*). No leaf malformation or any appreciable stunting of the plant was produced. Infected plants showed a typical leaf mottling, the dark-green tissue being most frequently found adjacent to the veinlets. Small streaks or islands of yellowish tissue later appeared but never to the extent to which they were produced by pea mosaic viruses 1 and 3. Leaf necrosis was not observed. The symptoms somewhat resembled those reported by Stubbs (11) for pea viruses 2B and 2C.

PEA MOSAIC VIRUS 5

Pea mosaic virus 5 (pea stunt mosaic) produced very similar symptoms on the Dwarf Telephone and Telephone varieties, but decidedly different symptoms on Green Giant.

On Dwarf Telephone and Telephone, stunting and dwarfing were characteristic features the plants reaching only about one-fourth natural size. Infected plants were only mildly mottled. The internodes were shortened, and the stem exhibited an intense purple discoloration followed by shrinking of the tissue. The vascular elements were discolored. About a week after inoculation the inoculated leaves were killed, and necrosis of the leaves about this region followed. Leaf necrosis was first evident at the base of the lamina, later becoming general over the entire leaflet. On Dwarf Telephone the growing point was often rosetted, the internodes were shortened, and the leaves were very compact, curled, and much smaller than normal (fig. 2, *A*). On both this variety and Telephone the terminal growth frequently became flaccid and died (fig. 2, *F*), and later the entire plant died. The leaves below the point of inoculation appeared normal.

On Green Giant only a slight stunting or leaf malformation was noted. Infected plants were decidedly mottled (fig. 2, *B*). In general, the lighter green tissue was found adjacent to the midrib and branch veins with darker islands adjacent to the veinlets. The light-green tissue later became yellow.

ALSIKE CLOVER MOSAIC VIRUS 1

The symptoms produced by alsike clover mosaic virus 1 were much the same on Dwarf Telephone, Telephone, and Green Giant varieties. The leaves were typically mottled and bore symptoms resembling those

of pea virus 3 except that they were slightly less intense (fig. 3, *C* and *D*). They were, however, more striking than those produced by pea mosaic virus 4 (fig. 1, *A* and *B*). Leaves of diseased plants were lighter green than normal, with small to fairly large, irregularly shaped dark-green patches of tissue usually surrounding the small veinlets; larger areas of yellow frequently appeared later. The symptoms were quite similar to those produced by pea virus 2B of Stubbs (11). Infected plants were only slightly stunted and only minor foliage distortion or reduction in size of leaves and stipules was noted.

ALSIKE CLOVER MOSAIC VIRUS 2

The symptoms produced by alsike clover mosaic virus 2 were distinctly different from and more severe than any thus far reported. They were quite similar on Dwarf Telephone, Telephone, and Green Giant, except that Dwarf Telephone was more stunted than the other varieties. Typical mottling such as was common with the three other viruses did not occur. The inoculated leaves usually died (fig. 4, *A*), as they did with the pea stunt virus. The leaves and stipules that arose at the first and sometimes the second node above the inoculated leaves, as well as those below the point of inoculation, remained normal, with the exception of an occasional leaflet exhibiting necrotic spots (fig. 4, *A* and *I*). Above this region the leaves were yellowish green and crinkled, and did not unfold so readily as normal ones. Infected leaflets were usually one-fourth to one-eighth normal size. The leaves arising from buds at the axils of the inoculated leaves, as well as offshoots arising from below the point of inoculation, showed the typical symptoms, which were later followed by the appearance of numerous small brown necrotic spots (fig. 4, *I*). Infected leaflets and stipules appeared water-soaked (fig. 4, *A* and *I*) and semitransparent. The green tissue was only found adjacent to the veins. These leaves finally died and fell off, leaving the bare stem and the malformed growing tip (fig. 4, *A*), which sometimes died later, leaving a few green normal-appearing leaves above the point of inoculation. The stems and petioles became very brittle and were readily broken. If pods were produced they were malformed and seriously spotted and pitted (fig. 4, *B*) and never reached maturity.

RESISTANCE AND SUSCEPTIBILITY OF PEA VARIETIES

Twelve varieties of peas were inoculated with each of the viruses studied. For the sake of comparison, the varieties selected were in many cases identical with those employed by other students of legume viruses.

It is seen from table 1 that Horal, Little Marvel, Perfection, Surprise, and Wisconsin Early Sweet are resistant to the four viruses, whereas the other varieties are susceptible to all of them. The symptoms produced by the several viruses differed in intensity, depending upon the virus used and the variety tested. Pea mosaic virus 5 and alsike clover mosaic virus 2, in general, produced the most severe symptoms; pea mosaic virus 4 and alsike clover mosaic virus 1 produced milder symptoms. In practically all cases a high percentage of infection occurred; however, alsike clover mosaic virus 2 produced only 50 percent infection in Harrison Glory and 70 percent in Laxton Progress (table 1).

TABLE 1.—*Susceptibility and resistance of 12 pea varieties to pea mosaic virus 4, pea mosaic virus 5 (pea stunt mosaic), and alsike clover mosaic viruses 1 and 2*

Variety	Reaction to --							
	Pea mosaic virus 4		Pea mosaic virus 5		Alsike clover mosaic virus 1		Alsike clover mosaic virus 2	
	Plants inoculated	Plants infected ¹	Plants inoculated	Plants infected ¹	Plants inoculated	Plants infected ¹	Plants inoculated	Plants infected ¹
	Number	Number	Number	Number	Number	Number	Number	Number
Alaska	10	10b	12	12a	10	10c	10	8a
Dwarf Telephone	10	10b	11	11a	10	10b	10	10a
Green Admiral	10	10b	10	10b	13	13c	15	15a
Green Giant	10	9b	13	12b	10	10b	10	10a
Harrison Glory	11	11c	10	9b	10	9b	14	7a
Horal	12	0	10	0	12	0	10	0
Laxton Progress	18	18b	10	9a	10	10b	17	12a
Little Marvel	10	0	11	0	10	0	10	0
Perfection	10	0	10	0	10	0	10	0
Surprise	10	0	26	0	10	0	11	0
Telephone	10	10b	15	15a	10	10b	10	10a
Wisconsin Early Sweet	10	0	22	0	22	0	22	0

¹ Degree of infection denoted by letters: a=serious, b=moderate, c=mild.

In comparing differences in infectivity between the viruses reported herein and those of other workers, it is evident (see table 7) that the four viruses described by the writer differ from pea virus 1 (pea enation mosaic), pea virus 2 of Osborn (6), pea virus 3 (common pea mosaic), bean virus 2 (white sweetclover mosaic), white clover mosaic virus 1, pea streak virus 1, alfalfa mosaic viruses 1, 1A, and 1B, and the vein mosaic virus of red clover. Pea virus 1 is infectious to the Horal, Perfection, and Surprise varieties (see table 7), but these varieties were not infected by the viruses described herein (table 1). Pierce (10) reported pea virus 3 as infectious to all of the above-named varieties except Horal, whereas Murphy and Pierce (4), working with the same virus, reported these varieties to be resistant, except a strain of Surprise. Pierce (10) found Alaska and Surprise susceptible to bean virus 2, and all the differential pea varieties susceptible to white clover virus 1. Osborn (7) found Horal and Perfection susceptible to a vein mosaic virus of red clover. Zaumeyer (14) reported that all four differential varieties were susceptible to pea streak virus 1 and that only Horal was resistant to three strains of the alfalfa mosaic virus. Stubbs (11) reported pea viruses 2A, 2B, and 2C infectious to Surprise but not to Perfection. It is evident from these facts that the four viruses discussed in this paper differ from the previously reported legume viruses in infectivity to certain pea varieties.

SYMPTOMS PRODUCED ON BEANS

PEA MOSAIC VIRUS 4

The symptoms produced by pea mosaic virus 4 on Stringless Green Refugee bean were very similar in certain respects to those produced by bean virus 2 (white sweetclover mosaic virus). Yellow halolike spots (fig. 1, *E* and *F*) appeared on leaves above the leaves that were inoculated, but the spots were less distinct and more diffuse than those produced by the sweetclover mosaic virus. The infected leaves drooped at the pulvini; later, the uppermost leaves became quite

severely mottled, crinkled, and puckered; and the young trifoliate leaves were sometimes decidedly malformed (fig. 1, *D*). In these respects the symptoms were quite similar to those produced by the common bean mosaic. Infected plants showed considerable stunting and frequently died. On Robust bean, the yellow spotting was not so pronounced but the mottling was very characteristic (fig. 1, *C*). Infected plants sometimes showed considerable stunting. The symptoms produced on Idaho Refugee bean and U. S. No. 5 Refugee bean were identical. The symptoms were similar at first to those noted on Stringless Green Refugee but later were less intense.

PEA MOSAIC VIRUS 5

Pea mosaic virus 5 (pea stunt mosaic), when inoculated on Stringless Green Refugee bean, produced symptoms that were very similar in many respects to those produced by white clover virus 1 on beans. The mottling was much milder than that produced by the mild pea mosaic virus but more intense than that produced by white clover virus 1 on beans. The older trifoliate leaves became slightly chlorotic and the mottling very blotchy with little malformation of the leaves (fig. 2, *D* and *E*). No appreciable stunting of the plants was noted. The symptoms were similar in all the susceptible varieties.

ALSIKE CLOVER MOSAIC VIRUS 1

The symptoms produced by alsike clover mosaic virus 1 on beans resembled those produced by pea mosaic virus 5. Yellow halolike spots were noted on the trifoliate leaves (fig. 3, *E*), but they did not appear so quickly and were not so intense as those produced by pea mosaic virus 5. The yellow chlorotic blotching, which was characteristic on the trifoliate leaves, sometimes eventually caused considerable chlorosis and appeared somewhat rugose. Infected plants were only slightly stunted. On Robust bean no chlorosis was observed, but mottling, puckering, and slight blistering of the leaves were characteristic (fig. 3, *B*). On Corbett Refugee bean the symptoms were slightly milder than those on the other varieties except Robust.

ALSIKE CLOVER MOSAIC VIRUS 2

Alsike mosaic virus 2 produced the most severe symptoms of any of the viruses. They were very similar to but more severe than those caused by pea mosaic virus 4. Yellow halolike spots appeared on the trifoliate leaves, and the leaflets later drooped at the pulvini. The youngest trifoliate leaves were mottled, puckered, and blistered, and smaller than normal (fig. 4, *C*, *D*, *E*, *F*, *H*). The internodes were shortened and the plants were extremely stunted. Later they became very chlorotic and many died. The symptoms were more severe and pronounced than those of the common bean mosaic. On U. S. No. 5 Refugee and on Robust the symptoms were not so severe as on the other susceptible varieties but in general they were similar.

RESISTANCE AND SUSCEPTIBILITY OF BEAN VARIETIES

The four viruses were inoculated on seven bean varieties, namely, Stringless Green Refugee, Corbett Refugee, Idaho Refugee, Wisconsin Refugee, U. S. No. 5 Refugee, Robust, and Great Northern U. I. No. 1. The results are shown in table 2.

Stringless Green Refugee, Idaho Refugee, U. S. No. 5 Refugee, and Robust were susceptible to pea mosaic virus 4, while Corbett Refugee, Wisconsin Refugee, and Great Northern U. I. No. 1 were resistant.

U. S. No. 5 Refugee was the only variety resistant to pea mosaic virus 5 (pea stunt mosaic). Great Northern U. I. No. 1 and Robust were very slightly susceptible to this virus, each having 1 infected plant out of 23 and 21 inoculated plants, respectively. It is probable that these were admixtures. The symptoms produced by pea mosaic virus 5 were milder than those produced by pea mosaic virus 4.

Great Northern U. I. No. 1 was resistant to alsike clover mosaic virus 1, while all of the other varieties were susceptible. Robust and Corbett Refugee exhibited the severest and the mildest symptoms, respectively.

All the varieties tested, except Great Northern U. I. No. 1, were susceptible to alsike clover mosaic virus 2. In general, the symptoms produced by this virus were more severe than those caused by any of the other viruses. U. S. No. 5 Refugee and Robust exhibited milder symptoms than any of the other varieties.

TABLE 2.—*Susceptibility and resistance of seven bean varieties to pea mosaic virus 4, pea mosaic virus 5 (pea stunt mosaic), and alsike clover mosaic viruses 1 and 2*

Variety	Reaction to—							
	Pea mosaic virus 4		Pea mosaic virus 5		Alsike clover mosaic virus 1		Alsike clover mosaic virus 2	
	Plants inoculated	Plants infected ¹	Plants inoculated	Plants infected ¹	Plants inoculated	Plants infected ¹	Plants inoculated	Plants infected ¹
	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>
Stringless Green Refugee	21	21a	10	10c	22	17b	20	20a
Corbett Refugee	11	0	9	9c	12	9c	9	5a
Idaho Refugee	11	5b	10	10c	11	8b	10	6a
Wisconsin Refugee	11	0	10	10c	10	6b	11	7a
U. S. No. 5 Refugee	12	12b	12	0	11	8b	12	4b
Great Northern U. I. No. 1	9	0	23	1c	12	0	11	0
Robust	11	8b	21	1c	12	12a	13	4b

¹ Degree of infection denoted by letters: a=serious, b=moderate, c=mild.

These results clearly show that these four viruses are unlike most of those reported earlier on legumes with respect to the susceptibility and resistance of certain bean varieties (see table 7). Pea virus 1, pea virus 3, pea streak virus 1, and vein mosaic virus of red clover are not infectious to bean, and in this regard differ from the four viruses discussed in this paper. Pea viruses 2A, 2B, and 2C of Stubbs (11) did not infect Stringless Green Refugee and Corbett Refugee, the only two varieties tested. Bean virus 2 and white clover virus 1, as shown by Pierce (10), infected all of the varieties listed in table 2 with the exception of U. S. No. 5 Refugee, which was not tested. In this respect these two viruses are similar to pea mosaic virus 5 (pea stunt mosaic), which infected all of the varieties tested except U. S. No. 5 Refugee. The percentage of infection with this virus (table 2) for Great Northern U. I. No. 1 and Robust was lower than that reported by Pierce (10) with white clover virus 1 and bean virus 2 for these same varieties. Furthermore, the symptoms produced by bean virus 2 were more severe than those produced by pea

mosaic virus 5. Those produced by white clover virus 1 and by pea mosaic virus 5 were of about equal intensity.

Pea virus 2 of Osborn (6) was found to infect Stringless Green Refugee, Corbett Refugee, and Robust, but not Great Northern U. I. No. 1. These varieties reacted similarly when inoculated with alsike clover mosaic viruses 1 and 2 (table 2). The symptoms produced by pea mosaic virus 2 on Stringless Green Refugee were not greatly unlike those manifested by alsike clover mosaic viruses 1 and 2 on this variety. These viruses produced a distinct mottling on the trifoliate leaves which was slightly more severe in the case of the alsike mosaic viruses than in that of pea virus 2. Osborn (6) reports a mild mottling on Robust, while alsike clover mosaic virus 1 produced a severe mottling and alsike clover mosaic virus 2 a mottling of moderate intensity. The alfalfa mosaic viruses 1, 1A, and 1B, according to Zaumeyer (14), produced local lesions on all of the bean varieties tested, whereas the four viruses described in this paper produced systemic infection.

The severe pea mosaic (see table 7) of Johnson and Jones (1) was reported to have infected all of the bean varieties except U. S. No. 5 Refugee, Wisconsin Refugee, and Idaho Refugee, which were not tested.

OTHER HOSTS

A relatively small number of hosts were inoculated with the four viruses to determine, if possible, differences in susceptibility and resistance. The results are shown in table 3. It is clearly evident that of these the susceptible hosts were restricted to the Leguminosae. It is also shown that, with few exceptions, hosts that were susceptible to one virus were susceptible to all four. *Crotalaria spectabilis* was susceptible to all the viruses except alsike clover mosaic virus 1. Pea mosaic virus 5 was the only virus infectious to *C. retusa*, while *C. striata* was susceptible to all the viruses. Only one plant of *Trifolium pratense* became infected with pea mosaic virus 4, and none with the other viruses. *Lupinus albus*, *L. angustifolius*, *Medicago sativa*, *Melilotus alba*, *Trifolium incarnatum*, and *Vicia faba* were susceptible to all of the viruses tested.

These data also show that the four viruses were dissimilar to the pea mosaic viruses reported by other investigators (see table 7). Pea virus 1, pea viruses 2A, 2B, and 2C, and pea virus 3 were not infectious to white sweetclover, differing in this respect from the four viruses discussed in this paper. Bean virus 2, pea virus 2, pea virus 3, white clover virus 1, the alfalfa mosaic viruses 1, 1A, and 1B, pea streak virus 1, vein mosaic of red clover, and severe pea mosaic were all infectious to red clover. Pea mosaic virus 4 was the only one reported here which infected this host. Pea mosaic virus 4 differed from bean virus 2, the three alfalfa mosaic viruses, and pea streak virus 1 in not being infectious to soybean. It was unlike white clover virus 1 and severe mosaic in that it was not infectious to white clover. The symptoms produced on red clover by the vein mosaic virus of red clover, according to Osborn (?), differed widely from those produced by pea mosaic virus 4.

TABLE 3.—*Susceptibility and resistance of various legumes and other plants to pea mosaic virus 4, pea mosaic virus 5 (pea stunt mosaic), and alsike clover mosaic viruses 1 and 2*

Host	Reaction to—							
	Pea mosaic virus 4		Pea mosaic virus 5		Alsike clover mosaic virus 1		Alsike clover mosaic virus 2	
	Plants inoculated	Plants infected ¹	Plants inoculated	Plants infected ¹	Plants inoculated	Plants infected ¹	Plants inoculated	Plants infected ¹
<i>Crotalaria spectabilis</i> Roth (No. 18096)	Number 4	Number 2a	Number 4	Number 2b	Number 4	Number 0	Number 4	Number 2a
<i>C. retusa</i> L. (No. 65331)	5	0	5	4b	5	0	5	0
<i>C. striata</i> DC. (No. 19322)	5	5b	5	5b	5	5b	5	5a
<i>Lupinus albus</i> L. (white lupine)	10	10a	10	10a	10	10b	10	10b
<i>L. angustifolius</i> L. (blue lupine)	10	9b	10	8b	10	8a	8	7a
<i>Medicago sativa</i> L. (alfalfa)	5	2c	5	3c			5	1c
<i>Melilotus alba</i> Desr. (white sweet-clover)	9	2b	9	6b	8	2b	9	4c
<i>Phaseolus aureus</i> Roxb. (mung bean)	10	0	10	0	10	0	10	0
<i>P. lunatus</i> L. (sieva bean, Henderson Bush variety)	11	0	10	0	10	0	10	0
<i>Soya max</i> (L.) Piper (soybean, Virginia variety)	10	0	10	0	10	0	10	0
<i>S. max</i> (L.) Piper (soybean, Manchuria variety)	10	0	10	0	10	0	10	0
<i>Trifolium incarnatum</i> L. (crimson clover)	5	5b	5	5a	5	5a	5	5a
<i>T. pratense</i> L. (red clover)	5	1b	5	0	5	0	5	0
<i>T. repens</i> L. (white clover)	9	0	9	0	9	0	9	0
<i>Vicia faba</i> L. (broadbean)	10	10b	10	9b	10	10b	10	10c
<i>V. sativa</i> L. (spring vetch)	10	0	10	0	10	0	10	0
<i>Datura stramonium</i> L. (jimsonweed)	10	0	10	0	10	0	10	0
<i>Nicotiana glauca</i> Graham	10	0	10	0	10	0	10	0
<i>N. glutinosa</i> L.	10	0	10	0	10	0	10	0
<i>N. tabacum</i> L. (Turkish tobacco)	10	0	10	0	10	0	10	0
<i>Petunia hybrida</i> Vilm. (petunia)	10	0	10	0	10	0	10	0

¹ Degree of infection denoted by letters: a=serious, b=moderate, c=mild.

PROPERTIES OF THE VIRUSES

Certain properties of the four viruses were determined in order to detect differences among them as well as to distinguish them from other known legume viruses. The thermal inactivation points, resistance to aging in vitro, and tolerance to dilution were determined. These studies were made with peas as the test host; however, the inoculum was not in every case prepared from infected pea plants.

Thermal inactivation point.—When infectious plant juice was heated for 10 minutes (table 4), the thermal inactivation points for the different viruses were as follows: Pea mosaic virus 4, 62° to 65° C.; pea mosaic virus 5 and alsike clover mosaic virus 1, 60° to 62°; and alsike clover mosaic virus 2, 54° to 58°.

Tolerance to dilution.—In regard to tolerance to dilution, pea mosaic virus 4, pea mosaic virus 5, and alsike clover mosaic virus 2 were still somewhat infectious at a dilution of 1 to 8,000 (table 5). Alsike clover mosaic virus 1 was infectious at 1 to 6,000, but not at 1 to 8,000.

Resistance to aging in vitro.—Pea mosaic virus 4 and alsike clover mosaic viruses 1 and 2 were no longer infectious after aging from 1 to 2 days (table 6). Pea mosaic virus 5 lost its infectivity in less than 1 day.

In general, the thermal inactivation points of the four viruses showed no outstanding differences from those of previously described legume viruses (see table 7). Pea mosaic viruses 4 and 5 were inac-

tivated at slightly higher temperatures than were white clover virus 1 and bean virus 2 (table 7), but the differences were not significant. Alsike clover mosaic virus 2 was killed at a somewhat lower temperature than the other viruses.

TABLE 4.—*Thermal inactivation points of pea mosaic virus 4, pea mosaic virus 5 (pea stunt mosaic), and alsike clover mosaic viruses 1 and 2, as determined by systemic infection of peas*

Temperature ¹ (°C.)	Reaction to—							
	Pea mosaic virus 4		Pea mosaic virus 5 (pea stunt mosaic)		Alsike clover mosaic virus 1		Alsike clover mosaic virus 2	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
	Number	Number	Number	Number	Number	Number	Number	Number
Not heated	20	18	23	15	20	20	25	25
54	27	13	30	7	30	22	29	5
58							25	0
60	29	6	30	2	27	2	30	0
62	27	1	30	0	30	0	30	0
65	25	0	25	0	30	0	30	0

¹ Virus was heated for 10 minutes at temperature indicated.

TABLE 5.—*Tolerance to dilution of pea mosaic virus 4, pea mosaic virus 5 (pea stunt mosaic), and alsike clover mosaic viruses 1 and 2, as determined by systemic infection of peas*

Dilution	Reaction to—							
	Pea mosaic virus 4		Pea mosaic virus 5 (pea stunt mosaic)		Alsike clover mosaic virus 1		Alsike clover mosaic virus 2	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
	Number	Number	Number	Number	Number	Number	Number	Number
None	24	23	20	17	22	22	20	13
1:2,000	12	9	15	2	16	5	9	7
1:4,000	12	8	29	4	16	5	12	5
1:6,000	33	9	30	1	31	1	32	4
1:8,000	30	2	30	1	30	0	30	1

TABLE 6.—*Resistance to aging in vitro of pea mosaic virus 4, pea mosaic virus 5 (pea stunt mosaic), and alsike clover mosaic viruses 1 and 2, as determined by systemic infection of peas*

Time aged at 20° C. (days)	Reaction to—							
	Pea mosaic virus 4		Pea mosaic virus 5 (pea stunt mosaic)		Alsike clover mosaic virus 1		Alsike clover mosaic virus 2	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
	Number	Number	Number	Number	Number	Number	Number	Number
0	20	18	20	20	20	18	10	10
1	23	17	20	0	26	5	26	5
2	20	0	20	0	22	0	26	0
3	20	0	20	0	22	0	20	0
4	12	0	10	0	12	0	12	0
5	12	0	10	0	12	0	12	0

Regarding longevity in vitro, pea mosaic virus 5 lost its infectiousness in less than 24 hours, which was a shorter time than was required by any of the other viruses except pea mosaic viruses 2A, 2B, and 2C, which were no longer infectious after $\frac{1}{2}$ to 1 day (table 7). Pea mosaic virus 4 and alsike clover mosaic viruses 1 and 2 remained infectious from 1 to 2 days, resembling in this respect bean virus 2 and pea streak virus 1. Pea virus 2, severe pea mosaic, white clover virus 1, and the alfalfa mosaic viruses remained viable longer than any of the other viruses listed.

In general, the four viruses reported herein were inactivated at higher dilutions than other legume viruses with the exception of pea streak virus 1, which lost its activity at about the same dilution, and pea virus 2, which was infectious at 1 to 100,000 dilution. Johnson and Jones (1) reported that severe pea mosaic was also infectious at a dilution of 1 to 100,000.

DISCUSSION

The data show that three of the viruses here described are different from previously described viruses that are infectious to pea. The fourth, alsike clover mosaic virus 1, is identical with a previously described mosaic virus from alsike clover (12). With the possible exception of alsike clover mosaic virus 2, these viruses produced symptoms on peas not greatly unlike those caused by certain other leguminous viruses. On particular varieties, such as Dwarf Telephone, pea mosaic virus 5 produced symptoms different from most of the other known viruses, except the alfalfa viruses which produced somewhat similar symptoms on this variety. It has been pointed out repeatedly that symptoms alone are not in general a dependable criterion for the differentiation of viruses but are of considerable help when coupled with other features.

Wherever possible the same varieties of peas, beans, and other hosts that were used by other investigators were employed in this study, and this made it possible to compare better the four viruses discussed in this paper with those previously described. In table 7 are listed a few of the differentiating features of most of the viruses that have been shown to be infectious to pea. All of the viruses were not tested on each host. The data on viruses other than the four discussed here were compiled from previous publications of the writer as well as from publications of other authors.

The four viruses described herein differed from most of the other legume viruses in not being infectious to Horal, Perfection, and Surprise pea varieties. Pea mosaic virus 2 was tested only on the Perfection variety, which was resistant. Pea mosaic virus 4 differed from the viruses that were infectious to bean in that it did not infect Great Northern U. I. No. 1 and Corbett Refugee (table 7). Pea mosaic virus 5 and alsike mosaic viruses 1 and 2 were not infectious to red clover, which distinguished them from the other legume viruses except pea virus 1 and pea viruses 2A, 2B, and 2C. They differed from these last-named viruses in that they were infectious to a number of bean varieties that were not infected by these viruses (table 7).

TABLE 7.—List of viruses affecting pea, showing differentiating features for the purpose of identification and classification ¹

Virus	Reaction of f—										Characteristics of virus								
	Differential pea varieties			Differential bean varieties						Other hosts		Physical properties	Dilution	Other characteristics					
	Alaska	Horai	Perfection	Surprise	Stringless (green Refu-ree	Robust	Great Northern U. L.	Corbett Refugee	Wisconsin Refugee	Idaho Refugee	U. S. No. 5 Refugee				Red clover	White sweetclover	White clover	Soybean	
Pea virus 1 (enation mosaic) (9, 10).	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	°C. 64-66	Longevity in vitro Days 4-5	1:10,000	Production of leaf enation on peas and crimson clover. Brilliant mottling of leaves of peas and crimson clover.
Pea virus 2 (8, 10).																62-64	4-5	1:100,000	
Pea viruses 2A, 2B, 2C (11).																62-64	1/2-1	1:1,000 to 1:2,000	
Pea virus 3 (common pea mosaic) (4, 10).	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	60-70 58-60	15 1-2	1:100,000 1:800 to 1:1,000	
Severe pea mosaic (1).																58	6-7		
Bean virus 2 (white sweetclover mosaic) (10).	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65-75	4-5	1:2,000 to 1:3,000	Considerable yellowing of leaves of beans and peas. Very mild mottling on leaves of peas and beans; necrosis of peas.
White clover virus 1 (10, 16).	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	62-65	1-2	1:2,000 to 1:4,000	Production of local lesions on bean leaves.
Alfalfa mosaic viruses 1, 1A, and 1B (14).	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	62-65	1-2	1:5,000 to 1:8,000	Intense streaking of stems of peas and terminal necrosis.
Pea streak virus 1 (14, 17).	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	58-60	2-3		Clearing of veins of peas, red clover, and 1 <i>trita jaba</i> .
Vein mosaic virus of red clover (7).	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	62-65	1-2	1:8,000 to 1:10,000	
Pea mosaic virus 4.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	62-65	0-1	1:8,000 to 1:10,000	
Pea mosaic virus 5 (pea stunt mosaic).	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	60-62 54-58	1-2 1-2	1:8,000 to 1:8,000 1:8,000 to 1:10,000	Severe stunting of Dwarf Telephone and Telephone pea varieties.
Alsike clover mosaic virus 1.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			1:6,000 to 1:8,000	Extreme leaf necrosis and drop of leaves of peas.
Alsike clover mosaic virus 2.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			1:8,000 to 1:10,000	

¹ For references to the reclassification of the legume viruses, see footnote 4.² Plus sign (+) represents infection; minus sign (-) represents no infection.

Zaumeyer and Wade (16) earlier reported an alsike clover mosaic virus which they named alsike clover mosaic virus 1. The symptoms that it produced on alsike clover, peas, and beans were very similar to those produced by alsike clover mosaic virus 1 herein reported. The only difference between the two viruses is that the earlier reported virus was slightly infectious to Perfection and Little Marvel peas, to which the virus of this study was not infectious. These differences might have been due to different strains or to admixtures of the varieties. In other respects the two appeared almost identical and hence are considered as the same virus. The alsike clover virus reported by Wade and Zaumeyer (12) is also identical with alsike clover mosaic virus 1.

The chief characteristic that differentiates pea virus 1 from the other viruses that are infectious to pea is the production of leaf enations on both peas and crimson clover, a symptom produced by none of the other legume viruses thus far described. Stubbs (11) reported that pea virus 1 produced severe top necrosis on seedlings of Dwarf Telephone pea. This is similar to the symptom produced by pea mosaic virus 5 on this variety.

Pea virus 2, described by Osborn (6), although not tested on all the hosts listed in table 7, differs from several of the viruses in its inability to infect Perfection pea. It differs from other viruses that infect this variety in the reaction to it of certain bean varieties. It most closely resembles alsike clover mosaic viruses 1 and 2, but these two viruses are not infectious to red clover which is susceptible to pea virus 2 (table 7). It also differs from these viruses in its ability to withstand aging longer in vitro. The symptoms produced by pea virus 2 on pea are markedly different from those produced by alsike clover mosaic virus 2 on this host.

Stubbs' pea viruses 2A, 2B, and 2C (11) closely resembled pea mosaic virus 3 in the symptoms and reactions that they caused to pea varieties (table 7). The principal points of difference were in the immunity of Perfection pea and the susceptibility of red clover to pea mosaic virus 3. Although red clover was not readily infected artificially with certain other viruses, it is probable that future studies may prove this host susceptible in some degree to these viruses. It is believed that these three viruses are possibly strains of pea mosaic virus 3.

Pea mosaic virus 3 was similar to bean mosaic virus 2 in its infectiousness to certain pea varieties but differed in not being infectious to bean (table 7).

Severe mosaic of peas, described by Johnson and Jones (1), was tested only on one of the four pea varieties listed in table 7, namely, Perfection, to which it was found to be infectious. It resembled white clover virus 1 in its host reaction, but the symptoms it produced on peas and beans were decidedly different from those produced by white clover virus 1. In certain of its properties this virus differed greatly from most of the legume viruses that have been reported as infectious to pea. Its longevity in vitro was 15 days, and it was still infectious at a dilution of 1 to 100,000 (table 7). None of the other viruses was viable beyond a dilution of 1 to 8,000, except pea virus 2. Johnson and Jones (1) considered severe pea mosaic similar to pea virus 3 and to white sweetclover mosaic. Since it does not infect beans it does not appear closely related to pea virus 3.

In host range, bean mosaic virus 2 most closely resembled the alfalfa mosaic viruses but differed markedly in the symptoms it produced on peas and beans. Their physical properties were also distinctly different (table 7). Perfection pea was immune to bean mosaic virus 2 but susceptible to the alfalfa viruses (table 7).

White clover mosaic virus 1 differed from the alfalfa mosaic viruses, which it most closely resembled, in the production of systemic infection in beans. The alfalfa mosaic viruses produced only local lesions on this host. Likewise, white clover mosaic virus 1 was infectious to Horal pea, while the alfalfa mosaic viruses were not. The thermal inactivation point for the alfalfa mosaic viruses was considerably higher than that for white clover virus 1.

Pea streak virus 1 differed from all of the other viruses in the fact that it produced a decided streaking of the stems of peas. The alfalfa mosaic viruses may also cause some streaking, but they differ from pea streak virus 1 in their ability to infect beans.

The vein mosaic of red clover reported by Osborn (?) most closely resembled pea mosaic virus 1, except that the latter was not infectious to red clover, white sweetclover, and white clover (table 7). Moreover, the symptoms produced by these two viruses on peas were unlike.

From the similarity in reaction of the several pea and bean varieties and other hosts to the four viruses discussed herein, it would appear that several of them are somewhat closely related. Likewise the physical properties of several of these viruses are quite similar. The thermal inactivation points, longevity in vitro, and dilution points for the four viruses dealt with in this paper were not sufficiently different to afford a basis for separating them. Table 7 shows that the thermal inactivation points of all of the legume viruses are approximately the same, and it is doubtful whether any of them could be separated by this means. Regarding longevity in vitro, the only viruses that were strikingly different from the four discussed here were pea virus 2, severe pea mosaic, white clover virus 1, and the alfalfa viruses. The four viruses discussed herein were infectious at higher dilutions than the other legume viruses except pea streak virus 1, which was still infectious at approximately the same dilution, and severe pea mosaic, which was reported by Johnson and Jones (1) as infectious at a dilution of 1 to 100,000.

Probably other new legume viruses will be described in the future. It is believed that such studies could be simplified by the employment by the different workers of standardized methods of technique and the use of the same differential varieties of peas, beans, and other hosts.

SUMMARY

Three new mosaic diseases infectious to pea, together with an earlier described mosaic virus from alsike clover, likewise infectious to pea, are described herein and identified.

The viruses studied were pea mosaic virus 4, pea mosaic virus 5 (pea stunt mosaic), alsike clover mosaic virus 1, and alsike clover mosaic virus 2. They were differentiated from one another and from the previously described legume viruses (1) by the symptoms they produced on peas and beans, (2) by the susceptibility and resistance of several varieties of pea, bean, and other legumes and of other hosts, and (3) by certain physical properties.

Pea mosaic virus 4 and alsike clover mosaic virus 1 produced comparatively mild symptoms on pea. Pea mosaic virus 5 produced considerable stunting of Dwarf Telephone and Tall Telephone pea varieties. Alsike clover mosaic virus 2 caused the most severe symptoms on peas, producing necrosis and leaf drop.

Twelve varieties of peas were inoculated with the four viruses. All were susceptible except Horal, Little Marvel, Perfection, Surprise, and Wisconsin Early Sweet.

The four viruses were infectious on certain bean varieties, but there were considerable varietal differences. Stringless Green Refugee was susceptible to the four viruses. Great Northern U. I. No. 1 was resistant to all of the viruses except pea mosaic virus 5. The viruses likewise produced different symptoms on the several varieties.

Pea mosaic virus 4 was the only one of the four viruses to infect red clover. The susceptible hosts were restricted to the Leguminosae. With few exceptions, hosts that were susceptible to one virus were susceptible to all.

Slight differences were noted between the physical properties of the four viruses affecting pea and those of viruses previously described. These differences were not sufficiently great to serve as the basis for definite identification and separation.

Certain important characteristic features of most of the viruses affecting pea are tabulated (table 7). The differences in most cases are sufficient for the purposes of differentiation and classification.

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PARTIAL SELF-INCOMPATIBILITY AND THE COLLAPSE OF FERTILE OVULES AS FACTORS AFFECTING SEED FORMATION IN ALFALFA¹

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INTRODUCTION

Various investigators have confirmed the fact, since Piper et al. (10)³ first reported it, that alfalfa (*Medicago sativa* L.) forms seed more freely after cross-pollination than after self-pollination. The authors (4) in a preliminary account, have shown that two probably distinct phenomena are involved. In the first place, it is found that alfalfa is partly self-incompatible. That is to say, male gametophytes are less effective in accomplishing fertilization in the plant from which they arise than in unrelated individuals. Moreover, there is a difference in survival of ovules containing inbred and hybrid embryos and endosperms, the former class being much more prone to collapse during development than the latter. The observations upon which these findings rest are presented in detail in the present paper and are considered in relation to the problem of seed setting in alfalfa.

Self-incompatibility is usually measured in terms of fruit and seed production. This may not be adequate in alfalfa in view of the possibility that ovule abortion, which also has a major effect on fruit and seed production, may be unrelated to self-incompatibility and may vary independently of it. The two phenomena have been separated in the present study by a histological study of the pistil following the two types of matings.

MATERIALS AND METHODS

Seven plants derived by self-pollination from six unrelated individuals selected from commercial varieties of alfalfa were used in the experiment. They were brought into flower in pots in a greenhouse at 70° to 80° F. in April, supplementary illumination being used to give a photoperiod similar to that in midsummer. The matings were made at the height of the flowering period. Newly opened flowers were castrated by clipping off the standard, forcing the sexual column out of the keel, and removing the pollen by suction. Some of the flowers were then selfed, using pollen from other flowers on the same respective plants. The remaining castrated flowers were cross-pollinated, the pollen being taken in each case from a different and unrelated plant within the group. The matings were so arranged that each of the plants, in addition to being selfed, served as a pistillate and as a staminate parent in the crosses. Intimate contact between the stig-

¹ Received for publication September 22, 1939. Contribution from the Department of Genetics (paper No. 247), Wisconsin Agricultural Experiment Station, University of Wisconsin.

² The authors desire to express their appreciation of the support received from the Wisconsin Alumni Research Fund and from the Work Projects Administration natural science project (No. 8649). They are also greatly indebted to Dr. Churchill Eisenhart, station statistician, for aid in the statistical analysis of the data.

³ Italic numbers in parentheses refer to Literature Cited, p. 472.

matic secretion and the pollen was assured by applying the pollen with toothpicks tipped with a strip of fine emery cloth.

The pistils were collected at 30, 48, 72, 96, 120, and 144 hours after pollination, fixed in Karpechenko's modification of Navashin's fluid, and embedded in paraffin. After the material had been sectioned and stained with Delafield's haematoxylin, the following data were taken on each pistil, regard being given to the serial order of the ovules from apex to the base of the ovary: Position of the most advanced pollen tubes, fertility of the ovules, stage of development of the pro-embryo or embryo, and fertile ovules collapsing. The technique employed did not permit of following pollen-tube growth in the style. Within the cavity of the ovary, however, the pollen tubes grow along the surface of the ventral suture, and, at this stage, the tips of the longest tubes can be distinguished clearly, although the distribution of the less advanced tubes cannot be determined accurately. About nine pistils per plant were taken at each collection in the self-pollinated series and about six pistils per plant in the crossed group.

SEED PRODUCTION FOLLOWING SELF- AND CROSS-POLLINATION

The comparative fertility on self- and cross-pollination of the seven plants used, measured in terms of mature seeds produced, is shown in table 1. The data are based on a separate series of matings made during the same period as the test pollinations. The flowers in the selfed series, however, were not castrated, and the pollen used in the crosses was derived mostly from unrelated plants not otherwise included in these experiments. It is not believed that either of these circumstances should influence the results significantly.

TABLE 1.—Fertility measured in terms of mature seeds produced, following self- and cross-pollination

Plant (pistillate parent)	Self-pollination				Cross-pollination			
	Flow-ers	Pods	Seeds	Seeds per flower	Flow-ers	Pods	Seeds	Seeds per flower
	Number	Number	Number	Number	Number	Number	Number	Number
A ¹	107	66	164	1.53				
B	74	26	43	.58	30	27	81	2.70
C	51	9	8	.16	24	22	133	5.54
D	105	45	78	.74	54	45	216	4.00
E	67	49	118	1.76	24	19	120	5.00
F	35	15	33	.94	76	69	450	5.92
G	43	15	27	.63	80	59	310	3.87
Total or average	375	159	307	.80	288	241	1,310	4.50

¹ Omitted from total.

Omitting plant A, for which the data are incomplete, it may be seen from table 1 that, on an average, about five and one-half times as many mature seeds developed per flower pollinated after crossing as after selfing. The values vary rather widely from plant to plant, particularly in the selfed series, but in the case of each individual a large increase is shown on crossing. This result is in conformity with the findings of Piper et al. (10), Frandsen (8), and Carlson (5).

The fertility differential between the crossed and selfed series is much wider when measured in terms of seeds formed per flower pollinated than in terms of pods set per flower pollinated. This follows

from the fact that the pods formed after selfing contained only 1.93 seeds each, on an average, whereas those in the cross-pollinated series contained 5.44 seeds.

OVULE FERTILITY

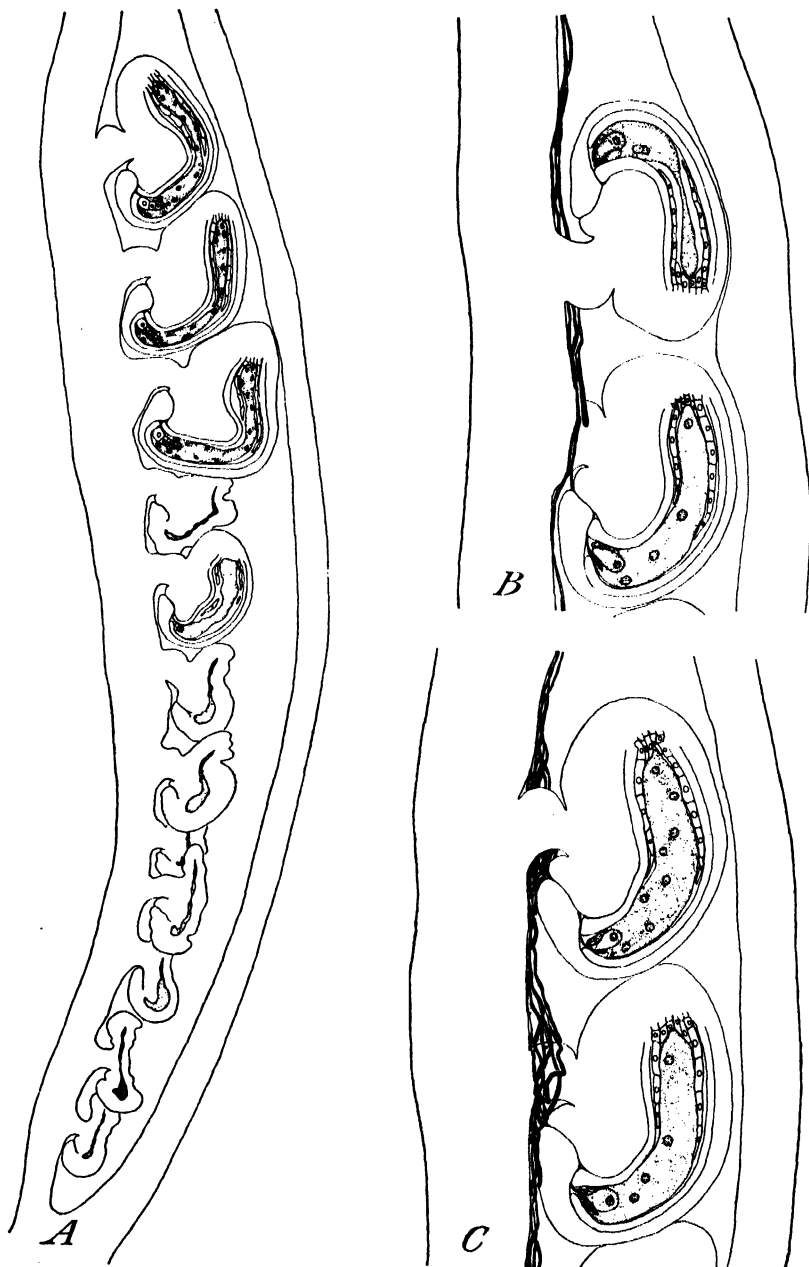
A primary cause of the disparity in the number of seeds that develop after self- and cross-pollination is found in the difference in the proportion of ovules that become fertile following the two types of matings. The ovary of alfalfa usually contains 10 to 12 ovules arranged alternately along the ventral suture in serial order. The ovules are comparatively uniform in size and, almost without exception, develop normal appearing embryo sacs. A necessary condition for the development of the seed is that fertilization of the egg within the ovule occur. A histological study of the ovary during the period immediately following completion of pollen-tube growth enables one to measure directly the frequency with which fertilization takes place. Failure of fertilization can thus be separated from other factors subsequently affecting fertility.

Just prior to fertilization the mature ovule is in a quiescent state. There is little, if any, evidence of nuclear or cell division in any of its parts. The pollen tube enters the embryo sac between the apices of the synergids and the egg. In the process of fertilization one male gamete nucleus fuses with the egg nucleus and the other unites with the fusing polar nuclei. Coincidental with fertilization active nuclear and cell division is stimulated in the integuments and funiculus and there is a considerable increase in the size of the ovule within a short period of time. The primary endosperm nucleus has oftentimes divided twice and there are four endosperm nuclei present at the time of the division of the zygote.

The fertilized ovules at 48 hours after pollination may be distinguished from those that have not been fertilized by the increase in size of the ovule, the development of the multinucleate endosperm, and the presence of a proembryo. If fertilization does not occur there is no further development of the ovule, the embryo sac collapses and ultimately the entire ovule, so that only a vestige of it remains at the later stages of the developing pod (pl. 1, A).

At 72 hours after pollination pollen tubes, which may be seen at certain earlier stages, can no longer be found within the cavity of the ovary. Fertilization, therefore, has ended, and a legitimate estimate of the frequency with which the process occurs after a given mating may be made. The results of an examination of ovaries collected at 72, 96, 120, and 144 hours after pollination are presented in table 2 and depicted graphically in figure 1. The samples collected at the different times provide the basis for four independent estimates of the percentage of fertile ovules in each plant in each of the two series.

It is evident from table 2 that a much larger proportion of the ovules become fertile after cross-pollination than after self-pollination. The respective mean values are 66.2 and 14.6 percent. Furthermore, the values for the two series are separated by a wide gap. The most fertile plant in the selfed series, B, shows 21.5 percent of the ovules containing a fertilized egg. The least fertile plant on crossing, which likewise proves to be B, has over twice as many fertile ovules. A statistical examination of the data brings to light additional relations not otherwise clearly apparent.



A, Median longitudinal section of an ovary 72 hours after self-pollination. The three apical ovules and the fifth ovule have been fertilized, the latter being in a stage of collapse. The remaining ovules have not been fertilized and are in stages of degeneration. $\times 425$. *B*, Median longitudinal section of a portion of an ovary showing the two apical ovules 30 hours after self-pollination. The second ovule has been fertilized. The pollen tubes are few in number. $\times 850$. *C*, Median longitudinal section of a portion of an ovary showing the two apical ovules 30 hours after cross-pollination. Both ovules have been fertilized. Pollen tubes are abundant, especially in the regions of the micropyles. $\times 850$.

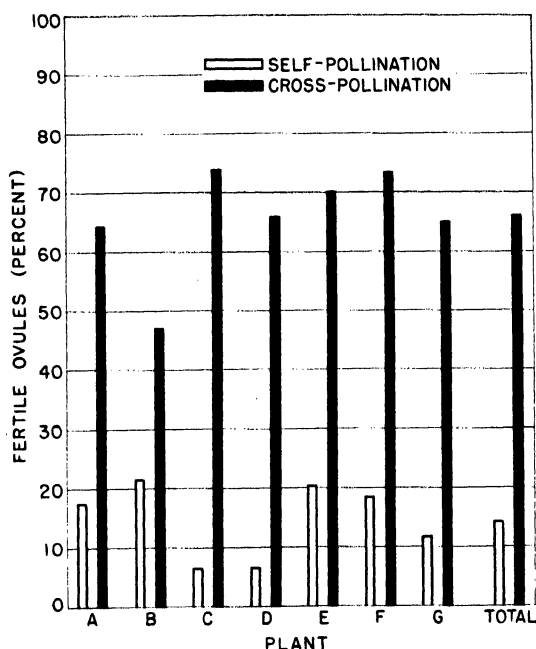


FIGURE 1.—Frequency of fertilization in individual plants following self-pollination and cross-pollination. Data based on pistils collected at 72, 96, 120, and 144 hours after pollination.

TABLE 2.—Comparative fertility of ovules in individual plants following self- and cross-pollinations; data based on collections at 72, 96, 120, and 144 hours after pollination

Plants selfed or crossed	72 hours			96 hours			120 hours			144 hours			Total		
	Total		Fertile	Total		Fertile	Total		Fertile	Total		Fertile	Total		Fertile
	No.	Pct.		No.	Pct.		No.	Pct.		No.	Pct.		No.	Pct.	
Selfed:															
A	58	10	17.2	82	21	25.6	58	4	6.9	12	2	16.7	210	37	17.6
B	44	14	31.8	58	10	17.2	26	5	19.2	44	8	18.2	172	37	21.5
C	76	10	13.2	79	4	5.1	82	5	6.1	60	1	1.7	297	20	6.7
D	82	5	6.1	73	2	2.7	72	6	8.3	20	4	20.0	247	17	6.9
E	65	9	13.8	43	15	34.9	47	6	12.8	37	9	24.3	192	39	20.3
F	136	29	21.3	167	28	16.8	153	25	16.3	130	27	20.8	586	109	18.6
G	127	10	7.9	137	12	8.8	125	23	18.4	54	10	18.5	443	55	12.4
Total	588		87	639		92	563		74	357		61	2,147		314
Crossed:															
A×B	58	34	58.6	81	53	65.4	77	52	67.5	73	48	65.8	289	187	64.7
B×C	58	25	43.1	61	26	42.6	60	30	50.0	54	29	53.7	233	110	47.2
C×D	56	38	67.9	77	68	88.3	48	28	58.3	48	37	77.1	229	171	74.7
D×E	71	43	60.6	52	32	61.5	53	38	71.7	82	58	70.7	258	171	66.3
E×A	50	34	68.0	44	32	72.7	54	37	68.5	57	41	71.9	205	144	70.2
F×G	92	67	72.8	81	54	66.7	50	40	80.0	86	67	77.9	309	228	73.8
G×F	90	78	86.7	92	55	59.8	72	38	52.8	50	27	54.0	304	198	65.1
Total	475		319	488		320	414		263	450		307	1,827		1,209

A reduction of the present data in original form by the analysis of variance technique might prove somewhat misleading in view of the fact that percentages have different variances depending on their values and the number of observations on which they are based. A more exact analysis may be made by first transforming the variates to θ values, according to the method proposed by Bartlett (1) and Bliss (2). This transformation has been made, and the analysis of variance procedure then applied. The analysis of variance based on the weighted θ 's corresponding to the observed percentages of fertile ovules is presented in table 3.

TABLE 3.—Analysis of variance based on weighted θ 's for fertility of ovules following self- and cross-pollination

Type of mating	Source of variance	Degrees of freedom	Sum of squares	Mean square	F	F _{.05}	F _{.01}
Self-pollination	Between plants	6	14.895378	2.4826	4.365	2.57	3.81
	Between replicates within plants	21	11.942734	.5687			
	Subtotal	27	26.838112				
Cross pollination	Between plants	6	13.805651	2.3009	3.245	2.57	3.81
	Between replicates within plants	21	14.890777	.7091			
	Subtotal	27	28.696428				

The first question of interest to which an answer may be sought is whether the increase in the proportion of fertile ovules following cross-pollination is actually larger than might arise from random sampling alone. Although the number of flowers used and the percentage of fertile ovules observed in the self- and cross-pollinated series vary rather widely, as may be seen in table 2, it is evident from table 3 that the estimated variances for the two groups, i. e., 0.5687 and 0.7091, are similar. The difference, in fact, is nonsignificant, since $F=1.25$, and for significance it would have to exceed 2.08. Consequently, these estimates may be pooled, giving 0.639 as the estimated variance of a single θ value. This corresponds to an estimated standard deviation of 0.709. The weighted mean θ 's for the two groups are as follows:

Self-pollination = 0.379, based on 2,147 ovules.

Cross-pollination = 0.956, based on 1,827 ovules.

The estimated standard deviation between these means, therefore, is 0.025, from which $t=23.1$. As this value of t far exceeds the 1-per-cent value (2.7) for 42 degrees of freedom, it may be concluded with considerable confidence that the self- and cross-pollinated groups differ significantly in proportion of fertile ovules.

The further question may be asked whether the frequency of fertile ovules is significantly increased by cross-pollination in the case of each plant. Since standard errors for the percentages are not available the significance of the differences must be tested on the basis of the mean θ values. These values for the several plants in the self- and cross-pollinated series are shown in table 4. The largest standard deviation for two mean values for the same plant, but for different series, is clearly less than $0.799\sqrt{\frac{1}{172} + \frac{1}{205}}$, so that an excessive mini-

mal significant difference is found by multiplying this by 2.704 (the 1-percent value for t , with 42 degrees of freedom). The resulting value is 0.223. Referring to table 4, it is seen that the smallest actual difference is 0.278 for plant B. Since this is significant it follows that the increase in percentage of fertile ovules on cross-pollination for each of the other plants is likewise significant.

TABLE 4.—Mean θ values and percentage of fertile ovules as estimated by inversion from the θ values for the individual plants in the self- and cross-pollinated series

Plant	Self-pollination			Cross-pollination		
	Total ovules	Weighted mean θ	Estimated fertile ovules	Total ovules	Weighted mean θ	Estimated fertile ovules
	Number		Percent	Number		Percent
A	210	0.423	16.8	289	0.935	64.7
B	172	.479	21.2	233	.757	47.2
C	297	.251	6.2	229	1.054	75.6
D	247	.254	6.3	258	.952	66.4
E	192	.459	19.6	205	.994	70.3
F	586	.445	18.5	309	1.035	73.9
G	443	.354	12.0	304	.950	66.2

A similar procedure may be followed in testing whether the different plants vary significantly in the proportion of ovules becoming fertile under each of the two systems of mating. It is found that the largest minimal 5-percent difference is 0.164 and the smallest minimal 5-percent difference is 0.100. Applying these minimal significant differences, it may be stated that, at the 5-percent level of significance, plants C and D are significantly less fertile than plants A, B, E, and F, and that the latter do not differ significantly from each other. Likewise, D does not differ significantly from C or from G. Furthermore, G is not significantly different from A and F. The exact minimal significant differences between G, on the one hand, and C, E, and B, on the other, are 0.118, 0.135, and 0.141, respectively. Plant G, therefore, does not differ significantly from any of these plants. The comparative fertility relations in the self-pollinated series may be summarized as shown in table 5.

TABLE 5.—Comparative fertility relations in self-pollinated plants A to G¹

Plant	A	B	C	D	E	F	G
A	0	0	+	+	0	0	0
B	0	0	+	+	0	0	0
C	—	—	0	0	—	—	0
D	—	—	0	0	—	—	0
E	—	—	+	+	0	0	0
F	0	0	+	+	0	0	0
G	0	0	0	0	0	0	0

¹ Plant A is significantly more fertile than C, and the latter is significantly less fertile than F. + denotes significantly greater, — significantly less, and 0 nonsignificance.

Proceeding similarly it may be shown that in the cross-pollinated series, plant B is significantly less fertile than the other individuals, and that these latter do not differ significantly from one another.

FERTILITY OF THE OVULES IN RELATION TO POSITION IN THE OVARY

A striking fact which is revealed by histological study of the pistils of alfalfa following self- and cross-pollination is a declining gradient in fertility of the ovules from the apex to the base of the ovary. The observations bearing on this point are summarized in table 6 and figure 2. In the selfed series, about one-third of the ovules in position 1,

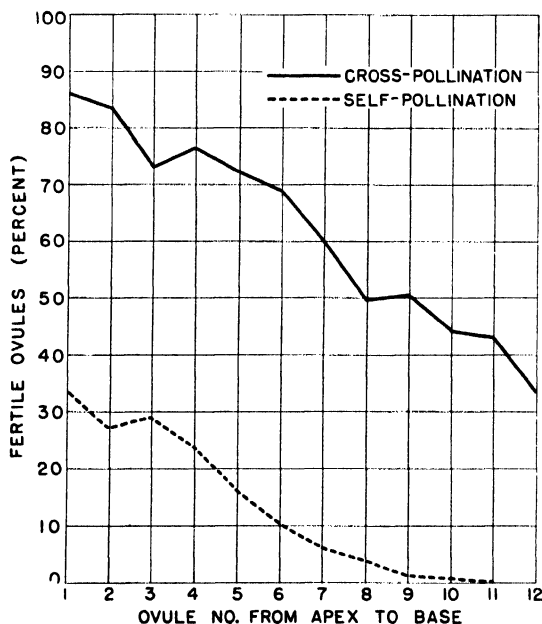


FIGURE 2.—Percentage of fertile ovules according to position in the ovary, following self- and cross-pollination. Averages based on pistils collected at 72, 96, 120, and 144 hours after pollination for seven plants. The apical ovule is No. 1.

that is, at the apex of the ovary, become fertile. Only about one-half as many in position 5 become fertile, and the frequency declines to zero towards the base.

The fertility gradient follows an almost parallel course in the cross-pollinated series, although the proportion of ovules becoming fertile in each position is much higher than after selfing. In position 1, for example, about 85 percent of the ovules contain fertilized eggs; in position 5 the value has declined to about 72 percent, and at the base of the ovary about one-third of the ovules become fertile.

POLLEN-TUBE GROWTH IN THE OVARIAN CAVITY

The explanation for the difference in the proportion of ovules that become fertile after self- and cross-pollination lies, in part, in the extent of pollen-tube growth following the two types of matings. As mentioned earlier, the position of the ends of only the most advanced pollen tubes within the ovarian cavity can be determined definitely by the technique used. This evidence, nevertheless, is sufficient to

show clearly that restricted pollen-tube growth is a factor affecting fertility after self-pollination.

TABLE 6.—*Fertility of the ovules in relation to position in the ovary following self- and cross-pollination*

[Data based on collections at 72, 96, 120, and 144 hours]

Ovary No. ¹	Self-pollination of ovules			Cross-pollination of ovules		
	Total	Fertile		Total	Fertile	
	<i>Number</i>	<i>Number</i>	<i>Percent</i>	<i>Number</i>	<i>Number</i>	<i>Percent</i>
1	205	70	34.1	181	156	86.2
2	205	56	27.3	181	152	84.0
3	205	59	28.8	181	133	73.5
4	205	49	23.9	181	139	76.8
5	205	33	16.1	181	131	72.4
6	205	21	10.2	181	125	69.1
7	205	13	6.3	181	109	60.2
8	205	9	4.4	181	90	49.7
9	191	3	1.6	159	80	50.3
10	172	1	.6	131	58	44.3
11	124	0	0	65	28	43.1
12	40	0	0	24	8	33.3
Total	2,167	314	14.5	1,827	1,209	66.2

¹ The apical ovule is No. 1.

The frequency distributions of pistils with reference to the farthest point of pollen-tube advance at 30 hours after pollination in the selfed and crossed series are shown in table 7. Position of the pollen-tube tips is recorded in terms of the particular ovule reached, 1 being the apical ovule. Length is expressed accordingly in "ovule units." The entries in the 0 column relate to pistils in which no pollen tubes were found within the ovary. There are two possible explanations for these cases. Either the pollen tubes were somewhat tardier in development and had not yet emerged from the base of the style, or the pistils had been so injured during castration and pollination that normal growth could not occur. It makes little difference in the conclusions to be drawn later whether these cases are included or omitted. Accordingly, they have been omitted from the summary columns.

It will be observed from table 7 that, in the case of each plant, the pollen tubes are farther advanced after crossing than after selfing. The difference, however, is much larger in some cases than in others. In plant D, selfed, for example, the longest pollen tubes have advanced on an average only slightly beyond the second ovule; whereas, in the cross-pollinated pistils from this individual, the longest tubes have reached the tenth ovule. The longest pollen tubes in plant G, on the other hand, are only slightly longer after hybridization than after selfing. There is little doubt, however, that the difference in length of the longest pollen tubes in the two series is statistically significant, as the comparison in the following paragraph shows.

TABLE 7.—Frequency distribution of pistils with reference to farthest point of pollen-tube advance at 30 and 48 hours after self- and cross-pollination, respectively

30 HOURS															
Mating	Pollen tubes penetrating to ovule No.--												Total pistils	Mean	
	0 ¹	1	2	3	4	5	6	7	8	9	10	11			12
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.		No.
A selfed	2				2	2	1							8	4.0
A × B	1													5	11.0
B selfed		3			1		1	2	2		1	3	1	9	4.8
B × C									3	5				8	8.6
C selfed	1			1	1	3		2						7	5.1
C × D	1			1							1	3	1	6	9.7
D selfed		3	2	2	2									9	2.3
D × E											6			6	10.0
E selfed					1		2	2	1	1				7	6.7
E × A							1		1	2	1			5	8.4
F selfed	2			2	3	3	1							9	4.3
F × G	1				1		1	1	1	1	3			8	8.0
G selfed	5	1	1		3			1	1					7	4.3
G × F	4			2	2	4	4	1						13	5.0

48 HOURS															
Mating	Pollen tubes penetrating to ovule No.--												Total pistils	Mean	
	0 ¹	1	2	3	4	5	6	7	8	9	10	11			12
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.		No.
A selfed					1	2	2	1	1					7	5.9
A × B														6	11.1
B selfed							2			5		1	4	7	8.1
B × C									1	4	2			7	9.1
C selfed				1		1	2	2						6	5.7
C × D												4	2	6	11.3
D selfed		1	1	1	2	1								6	3.2
D × E					1						2	4	1	8	10.0
E selfed									3	2	2			7	8.9
E × A									3	3	1			7	8.7
F selfed			2	1	2	5	5	2						20	5.4
F × G								1			4	1		6	9.7
G selfed	4			2	2	4	4	1						13	5.0
G × F	1										4	1		5	10.2

¹ Not included in summary columns; relates to pistils in which no pollen tubes were found within the ovary.

The weighted mean length of the longest pollen tubes of the seven plants in the selfed series is 414 ovule units, and the estimated variance is 13.75 ovule units (6 degrees of freedom). In the crossed series the corresponding values are 8.1 and 34.34. Since $F=2.50$, which is less than the 5-percent value, the two variances can be pooled, giving 24.04 as the estimated variance of a single mean. The standard deviation is, therefore, 4.90, and the standard deviation of the difference between the means of the two series is 0.93. It follows that $t=3.978$. Since this value of t exceeds that for the 1-percent point, 3.055, with 12 degrees of freedom, it may be inferred that at 30 hours after pollination the mean length of the longest pollen tubes in the crossed series is significantly greater than that in the selfed group.

An independent series of observations was made on the pollen tubes in the pistils collected at 48 hours after pollination. The data are presented in table 7. The results, in general, point to the same conclusion as was drawn above, namely, that following the application of pollen of an unrelated individual to a given plant the pollen tubes advance farther into the ovary than if the plant in question is self-pollinated. It will be noted, however, that for plant E, the difference, while very small, is in favor of the reverse relation.

With minor exceptions, the average length of the longest pollen tubes is greater at 48 hours than at 30 hours, following both self- and

cross-pollination. The average increase in length in the two series during this interval is 1.5 and 1.3 ovule units, respectively. It is not known whether the tubes continue to elongate beyond this period, for at the time of the next collection, 72 hours after pollination, they have disappeared. At 48 hours in the cross-pollinated series, the average lengths of the longest tubes indicate that the latter are already at or near the base of the ovarian cavity. In the selfed series, on the other hand, they are only slightly beyond the midpoint. Even if it be assumed that the pollen tubes in the selfed pistils continue to grow at the same rate during the 24-hour interval after 48 hours as in the preceding 18-hour interval it is evident that in the case of plants

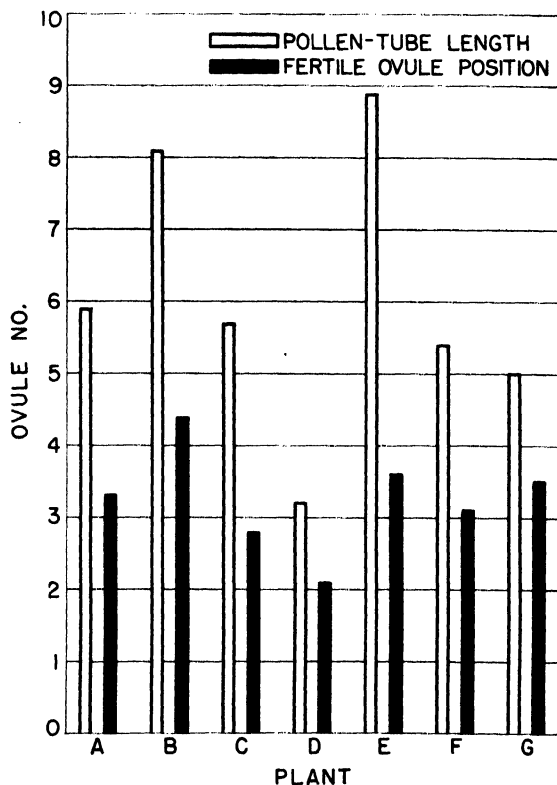


FIGURE 3.—Mean length unit of the longest pollen tubes at 48 hours after pollination in relation to mean position of the fertile ovules following selfing.

A, C, D, F, and G, at least, they would still fall short of reaching the basal ovules.

The mean length of the longest pollen tubes in the selfed series at 48 hours varies from 3.2 ovule units for plant D to 8.9 ovule units for plant E. It is of interest to know whether there is a parallel difference in the distribution of fertile ovules as determined from the collections made at, and following, 72 hours. The mean position of the fertile ovules in each plant has therefore been computed, and the values are presented in table 8 and figure 3 in relation to the corresponding data

for pollen-tube length. If the seven plants are now ranked in ascending order of magnitude for mean length of the longest pollen tubes, and in terms of mean position of the fertile ovules, the arrays shown in table 9 are obtained.

TABLE 8.—Mean length of longest pollen tubes at 48 hours in relation to mean fertile ovule position at 48, 72, 120, and 144 hours after self-pollination

Plant selfed	Mean length of longest pollen tubes	Mean position of fertile ovule	Plant selfed	Mean length of longest pollen tubes	Mean position of fertile ovule
A	5.9	3.3	E	8.9	3.6
B	8.1	4.4	F	5.4	3.1
C	5.7	2.8	G	5.0	3.4
D	3.2	2.1			

TABLE 9.—Array obtained when the seven plants are ranked in ascending order of magnitude for length of pollen tubes and position of fertile ovules

Character	1	2	3	4	5	6	7
Mean length of longest pollen tube	D	G	F	C	A	B	E
Mean position of fertile ovules	D	C	F	A	G	E	B

Several of the differences upon which the order depends are small and of doubtful statistical significance. Considering all the data, however, a positive relation is suggested between the two sets of observations.

Ovules which are not reached by pollen tubes after selfing obviously cannot become fertile. It does not necessarily follow, however, that the converse relation holds, namely, that ovules to which pollen tubes extend usually become fertile. There is little known concerning this phase of the self-incompatibility problem, so that the present data from alfalfa bearing on the question are of much interest.

It may be computed from the data in table 7 that at 48 hours after self-pollination the longest pollen tubes have reached at least to the apical ovule in 98.5 percent of the 66 pistils examined. The longest pollen tubes likewise extend to the second ovule, at least, in 94 percent of the cases. In other words, 48 hours after self-pollination the two ovules at the apical end of the ovary are reached by at least the longest pollen tubes in nearly all of the pistils. The percentages of fertile ovules in these two positions, on the other hand, are much lower. The value for position 1, as may be seen in table 6, is 34.1 percent, and that for position 2 is 27.3 percent.

In the cross-pollinated series at 48 hours the tips of the longest pollen tubes have passed the two apical ovules in all 45 pistils studied. In marked contrast with the low values after selfing, the percentages of fertile ovules in positions 1 and 2 in this group are 86.2 and 84.0, respectively. If it is shown that the two latter values are significantly higher than the corresponding percentages in the selfed series the operation of a factor affecting fertility in addition to pollen-tube length will have been demonstrated.

TABLE 10.—Summary of analysis of variance in terms of θ values for fertility of the first two ovules at the apex of the ovary following self- and cross-pollination

Item	Ovule No. 1		Ovule No. 2	
	Self-pollination	Cross-pollination	Self-pollination	Cross-pollination
Weighted mean of θ 's	0.620	1.221	0.536	1.177
Variance (6 degrees of freedom)	.414081	1.026656	.823016	.561734
Ovules, number	205	181	205	181
P ($F_{.05}=4.28$)	2.479	1.465	1.684	2.470
Pooled variance (12 degrees of freedom)	.720368	.692374	.733588	.433804
Standard deviation	.849	.832	.857	.659
Standard deviation of difference of 2 means (12 degrees of freedom)	.087	.085	.087	.067
t ($t_{.01}=3.055$)	6.908	7.541	5.552	8.567

In table 10 are summarized the results of the statistical analysis, the latter again being made on the variates transformed in terms of θ . Since both the t values are much larger than the 1-percent value it is inferred that there is a significantly higher ovule fertility associated with cross-pollination than with self-pollination in each of the first two positions in the ovary. Evidently pollen tubes which reach an ovule after selfing frequently fail to effect fertilization. This conclusion has been confirmed by direct observation of pollen tubes within the ovules after the two types of mating. There is evidence of marked stimulation of the tubes in the vicinity of a micropyle after crossing (pl. 1, *C*). Following self-pollination, on the other hand, pollen tubes frequently pass directly by the micropyle of an infertile ovule without showing any tendency to enter (pl. 1, *B*).

COLLAPSE OF FERTILE OVULES

The development of the fertile ovules arising from the two series of matings was followed up to 144 hours after pollination. The average number of cells in the hybrid embryos (including the suspensor) at this stage is about 18.0 and, in the inbred embryos, about 14.5. Not all the ovules in which development is initiated by fertilization, however, continue growth, even during the comparatively brief period of 6 days after pollination. One of the most significant facts the present investigation has brought to light is that the mortality of the ovules containing inbred embryos and endosperms is much higher than that of the ovules containing hybrid embryos and endosperms. It turns out, therefore, that not only are fewer eggs fertilized following self-pollination but the relative fertility associated with this type of mating is further lowered by the collapse of many of the ovules in which development starts.

The data on frequency of collapsing fertile ovules in the two series as found in the pistils collected at 72, 96, 120, and 144 hours after pollination are summarized in table 11 and figure 4. The means by which this class of ovules may be distinguished have been described earlier by Cooper, Brink, and Albrecht (6).

Of the 314 fertile ovules observed in the selfed series, 108, or 34.4 percent, were found to be collapsing within 6 days after pollination. The frequencies for individual plants vary from 20.5 percent for E to 51.4 percent for B. In the cross-pollinated series, on the other hand, only 7.1 percent of the 1,211 fertile ovules recorded were collapsing.

TABLE 11.—*Frequency of fertile ovules collapsing in seven plants after self- and cross-pollination*

[Data based on collections at 72, 96, 120, and 144 hours after pollination]

Plant	Fertile ovules			θ
	Total	Collapsing		
		Number	Number	
Selfed:				
A	37	9	24.3	0.515
B	37	19	51.4	.799
C	20	7	35.0	.633
D	17	7	41.2	.697
E	39	8	20.5	.470
F	109	39	35.8	.641
G	55	19	34.5	.628
Total	314	108	34.4	
Crossed:				
A \times B	187	13	7.0	.268
B \times C	110	5	4.5	.214
C \times D	171	13	7.6	.279
D \times E	171	16	9.4	.312
E \times A	146	9	6.2	.252
F \times G	228	14	6.1	.250
G \times F	198	16	8.1	.289
Total	1,211	86	7.1	

Furthermore, the percentage of aborting ovules of this class is uniformly low in all the plants. The difference in the mean values is highly significant statistically as the computations in the following paragraph show.

The weighted mean θ for the self-pollinated series is 0.624, based on

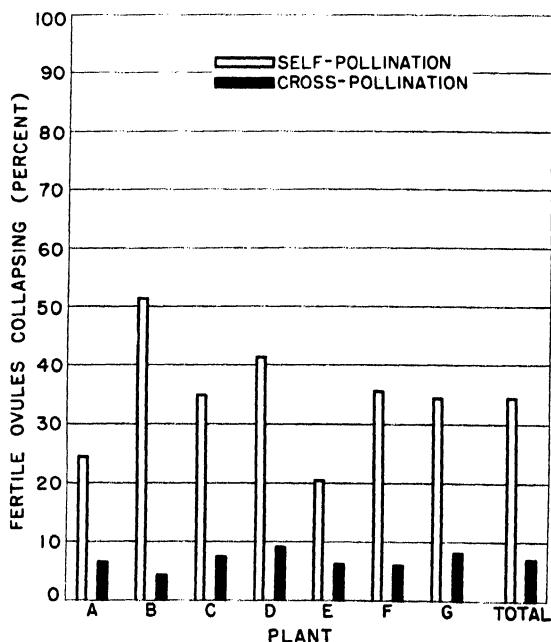


FIGURE 4.—Percentage of fertile ovules collapsing during the first 144 hours after self- and cross-pollination.

314 ovules, and 0.269 for the cross-pollinated group, based on 1,211 ovules. The standard deviation of the difference between the means of the two groups is 0.034. It follows that $t=10.44$, which is much larger than the value of t at the 1-percent point, namely, 3.055.

The relation between position in the ovary and frequency of collapse of fertile ovules is shown in table 12. It will be noted that, after selfing, the proportion of ovules in which development is arrested is comparatively high throughout. There is probably a tendency, however, for the frequency to increase somewhat in the lower positions. In the cross-pollinated series the values are fairly constant down to the ninth ovule, after which a small increase is shown. Uniform gradients similar to those associated with fertilization in the two series are not in evidence here.

TABLE 12. *Frequency of ovules collapsing in relation to position in the ovary following self- and cross-pollination*

[Data based on collections at 72, 96, 120, and 144 hours after pollination]

Ovule No. ¹	Self-pollination			Cross-pollination		
	Fertile ovules			Fertile ovules		
	Total	Collapsed		Total	Collapsed	
	Number	Number	Percent	Number	Number	Percent
1	70	18	25.7	156	12	7.7
2	56	16	28.6	152	13	8.5
3	59	17	28.8	133	3	2.2
4	49	20	40.8	139	11	7.9
5	33	12	36.4	131	12	9.2
6	21	10	47.6	125	10	8.0
7	13	10	76.9	111	7	6.3
8	9	4	44.4	90	3	3.3
9	3	1	33.3	80	3	3.7
10	1	0	0.	58	7	12.1
11				28	3	10.7
12				8	2	25.0
Total	314	108	34.4	1,211	86	7.1

¹ The apical ovule is No. 1.

FREQUENCY OF FERTILIZATION AND COLLAPSE OF FERTILE OVULES IN RELATION TO THE PRODUCTION OF MATURE SEEDS

The seven plants used in the experiment formed about 10.5 ovules per pistil, on an average (table 6). It is shown in table 1 that, per flower pollinated, 4.5 mature seeds were formed after crossing and 0.8 seed after selfing. To what extent is the difference between the potential and the actual fertility accounted for by the two factors measured, namely, failure of fertilization and collapse of fertile ovules?

Referring to table 2, it may be seen that 14.6 percent of the ovules became fertile after selfing and 66.2 percent after crossing. Of the ovules which became fertile 34.4 percent containing inbred embryos and endosperms collapsed within 6 days after pollination as compared with 7.1 percent containing hybrid embryos and endosperms (table 11). Considering these two factors alone, the expected average number of seeds per flower is 1.00 and 6.15 for the two respective types of mating (fig. 5). This means that under the conditions of the present

experiment about 98 percent of the difference between the potential and actual fertility following self-pollination and about 67 percent following cross-pollination are accounted for by the lack of fertilization and the collapse of fertile ovules during the first 6 days.

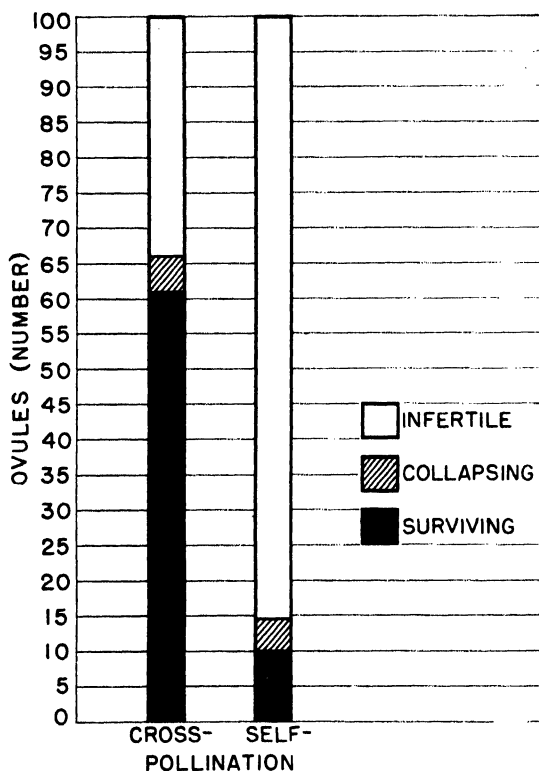


FIGURE 5.—Effect on fertility of failure of fertilization and the collapse of fertile ovules during the first 144 hours after pollination, following selfing and crossing.

DISCUSSION

In an earlier study of fertility in alfalfa in which the effects of self-pollination alone were determined, Cooper, Brink, and Albrecht (6) observed that (1) the pollen tubes frequently fail to reach the basal ovules, (2) many ovules remain infertile even though pollen tubes are present, and (3) abortion of fertile ovules is of common occurrence. These findings, which are confirmed in the present investigation, assume added significance in the light of the important additional fact now established that if cross-pollination to an unrelated individual is substituted for selfing the fertility is greatly increased. Crossing was found to raise the proportion of ovules becoming fertile from 15 percent to 66 percent, on an average, and to reduce the abortion of fertile ovules during the first 144 hours after pollination from 34 percent to 7 percent. The higher ovule fertility is found to result from more extensive growth of the pollen tubes within the cavity of the ovary and an increased tendency for the pollen tubes which reach the ovules to

enter the micropyles and accomplish fertilization. These facts make it clear that one of the basic phenomena involved in reproduction in alfalfa is partial self-incompatibility.

About five and one-half times as many mature seeds were formed per flower pollinated after crossing as after selfing in the seven plants used in the present experiment. On the basis of the values given in the preceding paragraph for frequency of fertilization and survival of fertile ovules up to 144 hours after pollination, crossing would be expected to increase the fertility about sixfold. Some additional ovules probably collapse after 144 hours, so that an exact correspondence between the two ratios is not to be anticipated. The fact, however, that the values are of the same order of magnitude lends further weight to the conclusion that the two factors identified are mainly responsible for the increased seed formation on cross-pollination.

Significant differences between individuals were found in the proportion of ovules becoming fertile after self-pollination. The extreme values within the seven plants examined were 6.7 and 21.5 percent. In a previous investigation, Cooper, Brink, and Albrecht (6) found one plant in which 43.7 percent of the ovules became fertile after selfing. Doubtless study of larger populations would disclose an even wider spread. The conclusions that self-incompatibility in alfalfa is only partial and varies considerably from plant to plant are in accord with the writers' observations on fertility in larger numbers of individuals being selfed in a breeding experiment by hand-tripping of the flowers. Rarely is a plant found on which no seed is formed after selfing, although the yield is often low. Occasional individuals occur in which the self-fertility is relatively high. It is not known in these latter cases whether it could be increased by applying pollen from unrelated plants. All seven plants in the present experiment, however, showed a statistically significant increase in percentage of fertile ovules on crossing in spite of considerable variation in degree of self-incompatibility.

The abortion of fertile ovules, which occurs so freely following self-pollination, may be a manifestation of self-incompatibility per se, or it may be an inbreeding effect. That is to say, either some of the genes conditioning pollen-tube behavior also may tend to cause ovule collapse, or recessive genes at other loci, adversely affecting early development of the zygote and brought together in homozygous condition by the inbreeding, may be responsible. When the data in table 2 showing the percentage of ovules becoming fertile after self-pollination in the seven plants are compared with those in table 11 showing the frequency of fertile ovules collapsing in the same individuals, no correlation is apparent between the two sets of values. Examination of the data obtained by Cooper, Brink, and Albrecht (6) on another series of alfalfa plants leads to the same result. While the evidence is rather limited, it indicates that the collapse of fertile ovules after selfing is more likely an effect of inbreeding than a direct manifestation of self-incompatibility.

The fact is well known to those who have had an opportunity to observe it that alfalfa is highly variable in reproductive behavior. The plant, although blossoming freely, rarely sets seed in many geographical areas in which it is satisfactorily grown as a forage crop. Even in regions where seed is produced commercially, the yield may

fluctuate widely. The reasons for the variability are not yet sufficiently well understood to permit of a general interpretation of this behavior. Meanwhile, however, the implications of partial self-incompatibility may be examined.

The following two deductions of direct significance for an understanding of seed formation in alfalfa may be drawn from the evidence presented above: (1) The potential fertility of a plant will usually, if not always, fall far short of complete realization if self-pollination alone prevails. Under uniform environmental conditions the degree of self-incompatibility varies widely from plant to plant in alfalfa, as it does in many other self-incompatible species (11). The fertility of the ovules was markedly higher following cross-pollination, however, in each of the cases in which a direct comparison was made in the present study. The degree of self-incompatibility of a given genotype may also vary according to external conditions judging from the behavior of other self-incompatible species (11); but whether the environment can ever shift the reaction of an alfalfa plant to its own pollen so that the usual advantage of crossing disappears remains to be shown.

(2) If, on the stigmas of a given plant, the latter's own pollen is present along with that from an unrelated plant, under conditions favorable for germination, the pollen from the unrelated plant will probably have a large advantage in effecting fertilization. This point is of particular significance for alfalfa since in conformity with the structure of the flower, the stigma usually receives a copious amount of pollen from the anthers encircling it even before the flower opens. The validity of this conclusion rests on (a) the observed fact that, following crossing, the pollen tubes not only grow more rapidly and extend farther into the ovarian cavity than after selfing but they also show a greater tendency to enter the micropyles and effect fertilization in the ovules reached and on (b) the applicability to alfalfa of the rule East and Park (7) established for self-incompatible tobaccos that mixed self- and cross-pollination does not increase self-fertilization.

The discovery of partial self-incompatibility brings into sharp relief another phase of the fertility problem in alfalfa, namely, the means by which pollen produced by one plant gains access to the stigma of another. Carlson (5) and Hadfield and Calder (9) have observed pollen on the standards of untripped flowers in the field. Doubtless much of this is brought from other plants by air currents and insects. When a flower trips, some of the pollen on the standard may be captured by the stigma and cross-pollination thus be effected. The question becomes perplexing, however, when regard is given to the findings of Carlson (5) that, under Utah conditions, alfalfa commonly sets seed without tripping of the flowers. Brink and Cooper (3) found the same conditions prevailing under unusually hot and dry weather at Madison, Wis., in 1936. Possibly the degree of self-incompatibility is lowered by the conditions of growth in these cases and much self-fertilization occurs. It seems more probable, however, that pollen from other plants gains access to the stigmas of untripped flowers by some means not yet described. Brink and Cooper (3) observed pollen germinating within untripped flowers and pollen tubes entering the styles, even in the late bud stage. Almost certainly the pollen functioning in the latter cases was from the same respective flowers. Conceivably, this

early self-pollination initiates wilting of the corolla and stimulates development of the pistil so that the stigma becomes exposed to pollen from other flowers. Since after selfing, the pollen tubes grow more slowly and fail to fertilize the eggs in many of the ovules, the pollen from other individuals might largely prevail in fertilization in spite of its later start. Obviously, this question requires further investigation.

SUMMARY

Following self-pollination under greenhouse conditions only 14.6 percent of the ovules of alfalfa become fertile, on an average, as compared with 66.2 percent after cross-pollination on the same plants.

Individuals vary significantly in the proportion of ovules that become fertile after selfing. The variability is less after cross-pollination.

Most of the fertile ovules resulting from self-pollination are in the apical half of the ovary. A rather uniform gradient in fertility occurs from the apex downwards, the ovules in the lowermost positions rarely containing fertilized eggs.

A parallel gradient in fertility is found after cross-pollination, although the proportion of ovules becoming fertile in each position is much higher than after selfing.

Pollen-tube growth, as determined by the position of the tips of the longest pollen tubes at 30 hours and at 48 hours after pollination, is more rapid in cross matings than after selfing.

It is probable that the basal ovules in the ovary are seldom reached by pollen tubes after selfing. Following cross-pollination the tubes usually grow to the base of the ovary.

In the self-pollinated series there appears to be a positive, although not high, correlation between mean length of the longest pollen tubes and mean position of the fertile ovules from plant to plant.

The low fertility of the ovules following self-pollination is due only in part to restricted pollen-tube growth. Direct observation confirms other, more general, evidence adduced that frequently after selfing the pollen tubes pass directly by the micropyles of ovules containing unfertilized eggs.

About one-third of the fertile ovules in the selfed series were found to be collapsing in the pistils collected at 72 to 144 hours after pollination as against only 7.1 percent in the crossed series.

Taking into consideration the differentials both in proportion of ovules becoming fertile and proportion of fertile ovules collapsing, the net fertility at 144 hours after pollination is about six times as high in the crossed series as in the selfed series.

The partial incompatibility associated with self-pollination and the high incidence of abortion of ovules containing inbred embryos during the first 6 days after pollination appears sufficient to account for most of the difference in seed production following selfing and crossing.

Under the conditions of the present experiment about 98 percent of the difference between the potential and actual fertility following self-pollination and about 67 percent following cross-pollination are accounted for by the frequency of fertilization and the collapse of fertile ovules during the first 6 days.

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SOME FACTORS IN LAMB PRODUCTION ASSOCIATED WITH SIZE AND TYPE IN MUTTON SHEEP¹

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INTRODUCTION

Among the different breeds of sheep there are marked differences in scale, weight, conformation, and fleshing qualities as well as in wool production. The factors of rapid growth and desirable mutton type receive primary consideration from most growers of the mutton breeds, whereas breeders of fine-wool sheep place great emphasis on the fleece. Western range men endeavor to maintain heavy wool-producing qualities in the ewe flock with a predominance of fine-wool breeding while raising an acceptable lamb for slaughter or feeding purposes by the use of various breeds of mutton-type rams. Farmers in the Central West desiring to purchase breeding ewes usually find greater numbers and more uniform selection among western ewes than among native ewes, and consequently they are confronted with the problem of the type and breed of ram to use.

Considerable data are available on the various methods of breeding for early spring lambs in the East (7, 9, 16),³ for early California lambs to be shipped to New York and other eastern markets (13), and for the production of the large western lamb crop (1, 8, 10, 11); yet there is little information on certain phases of lamb production in the Central West⁴ (2, 3). Few records have been made of comparative feed consumption of large and small ewes and their lambs in the course of the year⁵ (9, 10, 15), as practically all data are computed on a per lamb or per ewe basis with little or no regard to returns for feed consumed. In fact, as Hunt (9) points out in some of the tests referred to, if feed consumption were on the approximate basis of weights of the ewes, the interpretation of results quoted by the authors might be doubtful, or in many cases reversed. Records of carcass yields, including boned edible meat, for various types and sizes of lambs, are also lacking. Because of this scarcity of data a study was made at the Illinois Agricultural Experiment Station in which various factors in lamb production associated with size and type of sheep were considered.

¹ Received for publication October 21, 1939. This paper is part of a thesis presented to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of doctor of philosophy. The work herein described was done at Illinois while the author was on leave of absence from the Michigan station. The paper is presented as a contribution from the Illinois Agricultural Experiment Station.

² The author is especially indebted to Prof. H. P. Rusk of the University of Illinois for suggestions and guidance in the conduct of this study. Grateful acknowledgment is also made for the generous cooperation and assistance in the experimental procedure by members of the following divisions in the Department of Animal Husbandry, Animal Nutrition, Meats, and Sheep Husbandry. Additional assistance in certain phases through the Work Projects Administration Project No. 80057 is likewise appreciated.

³ Italic numbers in parentheses refer to Literature Cited, p. 486.

⁴ KISER, O. M., CHRISTGAU, R. J., and WINTERS, L. M. SUMMARY OF SHEEP BREKEDING INVESTIGATIONS AT THE NORTH WEST SCHOOL, CROOKSTON, 1934-37. Minn. Agr. Expt. Sta. Mimeographed Rpt. 1937.

⁵ See footnote 4.

OBJECTS OF INVESTIGATION

In these tests one of the largest mutton breeds, the Hampshire, was compared with one of the smallest mutton breeds, the Southdown. Other tests were made in which rams of the two breeds were mated with similar groups of western ewes. In these cases, rams of a breed intermediate in size, the Shropshire, were also used.

The objects of the investigation may be summarized as follows: (1) To determine the comparative total feed requirements for the production of market lambs from sheep of large and small mutton breeds; (2) to compare the digestive efficiency of sheep of the different groups; (3) to study methods of measuring meat values in lambs; (4) to compare yield and quality of meat from lambs of the two groups; (5) to compare the efficiency of rams of different size and breed for siring market lambs from grade western ewes under Central States conditions; and (6) to compare fine-wool type and crossbred-type western ewes under such conditions.

TESTS WITH PUREBRED LAMBS OF LARGE AND SMALL
MUTTON BREEDS

Twenty Southdown ewes were purchased in Kentucky, 10 from each of two breeders. Five ewes were 2-year-olds and the others were yearlings. Twenty Hampshire ewes were purchased at the stockyards in Evansville, Ind., where they had been sent for sale from a ranch in Utah. They were 3 years old. The ewes in each group were purebred and were considered by members of the Animal Husbandry Department to be typical of the two breeds. The average weight of the Southdown ewes was 109 pounds and that of the Hampshire ewes 153 pounds. The lightest Southdown ewe weighed 94 pounds and the heaviest 123, while the lightest Hampshire weighed 140 pounds and the heaviest 175.

The ewes were mated with purebred rams of the respective breeds, each of which had proved to be a good sire in the university flock. The Southdown ram was 6 years old, weighed 149 pounds, and was considered by sheepmen to be typical of the Southdown breed. The Hampshire ram was similar in age, of good type, and weighed 230 pounds.

METHOD OF PROCEDURE

Feed records were kept on each group of ewes from September 7, when breeding started, until the lambs were slaughtered. Feed for the dry period, to complete the yearly record after the lambs were slaughtered, was estimated on the basis of that required to maintain body weight during the first 8 weeks of pregnancy. Corn silage and alfalfa hay constituted the ewe ration, except during the latter part of pregnancy and during the nursing period, when grain was added. A mixture of bonemeal, limestone, and salt was available.

All lambs were born between January 30 and March 7, 1938, the average birth date of the Southdown lambs and of Hampshire single lambs being February 17, and that of Hampshire twin lambs February 14. For various reasons, such as death of ewes or of lambs, injury, failure of milk supply, or slaughter of finished lambs, it was necessary to remove individuals from time to time; and the feed was calculated

on the per head basis for each 14-day period. There were 14 Hampshire twins, 7 single lambs, and 14 Southdown single lambs on which complete feed data were obtained by lots.

A creep was arranged in each pen so that the lambs could go to the feed as they desired, although it was not allowed to accumulate before them. The same mixture, composed of corn, oats, and linseed meal, was fed each lot. The feed mixture was changed from time to time by increasing the corn content and later adding some cut alfalfa hay. The lambs ate some feed with the ewes in addition to that offered in the creep. Since all rations were from the same feeds in similar proportions, repeated chemical analyses were not made; however, one analysis was made of representative feeds used. The total digestible nutrients were estimated from Morrison's tables (14) for the kinds of feed used. This was done to convert all feeds to one unit of measure for approximate comparisons.

Time of reaching market finish, and average monthly changes in lamb prices by grades for 3 years, based on reports of the Bureau of Agricultural Economics of the United States Department of Agriculture, were considered in connection with the other factors. Previous shearing dates for the ewes were unknown, making study of wool production impossible.

The lambs were slaughtered when they reached a choice market finish, as judged by appearance and handling qualities. Physical and chemical analyses of the carcasses were made for two reasons, namely, to determine whether slaughter had been done when the two groups of lambs were similar in finish and to measure differences in the yield and quality of meat. Content of the digestive tract and weights of body parts were obtained at slaughter. The various carcass cuts were made and the right half of each carcass was separated with the knife into bone, lean, and fat. Some measurements were made to determine the relation of size of eye muscle in the loin and rib cuts to quantity of lean in the carcass. Measurements of color of lean in the rib eye were made with a spectrophotometer, showing the dominant hue or primary color, with its degree of purity and brightness. Chemical analyses were made of dry matter, ether extract, and total nitrogen in the total lean and total fat of the half carcass. Measurements of color were made by the Division of Meats and chemical analyses by the Division of Animal Nutrition, of the University of Illinois.

No data could be found to prove or disprove, among other things, the frequently expressed opinion that larger sheep handle roughages more efficiently than smaller sheep. Consequently, trials were conducted with six sheep of each breed 12 months old to compare efficiency in digestion of a full-fed ration of equal parts of grain and hay. The average weight of the Southdown sheep was 82.7 pounds, and that of the Hampshires 135.3 pounds.

Digestion data were also obtained with 20 pairs of Hampshire-sired and Southdown-sired grade lambs from this experiment. These lambs were fed at or slightly above maintenance level on approximately equal parts of grain and hay. The weights of the pairs were more nearly alike than in the test with purebred sheep. These tests were conducted in the animal nutrition laboratory in digestion crates similar to those described by Forbes (6). Collections were made over 10-day periods after preliminary periods of 10 days.

RESULTS

Statistical analyses of data were made according to Fisher's (5) method for small numbers, in which values for P of 0.01 or less are considered highly significant and values between 0.01 and 0.05 are considered significant.

Purebred Hampshire ewes, averaging 153 pounds in weight when bred, maintained their weights in a similar manner to purebred Southdown ewes averaging 109 pounds, when fed 50 percent more feed than was allowed the Southdown ewes during the first 8 weeks of pregnancy. Hampshires showed a stronger tendency to increase feed consumption and to gain in body weight when full-fed similar rations during the remainder of the pregnancy period, although fatness was similar in the two groups. Average weights before lambing were 170 and 112 pounds respectively, or approximately 50-percent greater weight for the Hampshire ewes than for the Southdown ewes. Among the 20 Hampshire ewes, there were born later 9 sets of twin lambs and 1 set of triplets, whereas there were only 2 sets of Southdown twins from the 20 Southdown ewes.

For maintenance during the year and for developing a desirable market lamb weighing 86 pounds, a Hampshire ewe and single lamb ate about 50 percent more alfalfa hay and corn silage and practically the same quantity of concentrates as did a Southdown ewe and lamb in developing a lamb weighing 66 pounds (table 1).

TABLE 1.—*Feed consumed by purebred ewes and lambs during various periods and for the year*

Lambs and period	Alfalfa hay	Corn silage	Shelled corn	Oats	Wheat bran	Linseed meal
Southdown ewe (average of 14; single lamb):	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
Breeding and pregnant, 168 days	241	304		21		
Nursing, 156 days	214	329	137			22
Dry, 41 days	62	62				
Total for the year (ewe)	517	694	137	21		22
Lamb	27		70	37	4	14
Total for ewe and lamb	544	694	207	58	4	36
Hampshire ewe (average of 7; single lamb):						
Breeding and pregnant, 168 days	364	471		35		
Nursing, 110 days	220	432	160			25
Dry, 87 days	196	196				
Total for the year (ewe)	780	1,099	160	35		25
Lamb	14		43	34	5	11
Total for ewe and lamb	794	1,099	203	69	5	36
Hampshire ewe (average of 7; twin lambs):						
Breeding and pregnant, 168 days	364	471		35		
Nursing, 152 days	330	523	203			33
Dry, 45 days	101	101				
Total for the year (ewe)	795	1,095	203	35		33
Lamb (1)	32		88	51	6	19
Total for lamb and one-half ewe total for the year	429	547	189	68	6	30

The calculated total digestible nutrient content of the total feeds eaten per ewe and lamb was approximately 30 percent higher for the Hampshire ewe and single lamb than for the Southdown ewe and lamb, as indicated in table 2.

TABLE 2.—*Total digestible nutrient requirement for purebred ewes and lambs, per lamb and per unit of body weight, including the ewe's requirement for the year*

Item	Average feed-lot final weight ¹	Total digestible nutrients per—			
		Lamb	Hundred- weight of final weight	Hundred- weight of chilled carcass	Hundred- weight of carcass lean
	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
Southdown single	65.57	643	980	1,960	3,608
Hampshire single	85.93	848	987	2,014	4,143
Hampshire twin	84.43	551	653	1,396	2,556
Average Hampshire lamb from ..					
1 single, 2 twins, or 150 percent crop ..	84.93	650	764	1,602	3,085
2 singles, 1 twin, or 120 percent crop ..	85.43	749	876	1,808	3,614

¹ Averages of 14 Southdowns, 7 single, and 14 twin Hampshires.

The average slaughter weight of Southdown lambs at an average age of 164 days was approximately 20 pounds lighter than that of Hampshire lambs when 117 days of age, although the finish was fully as good if not slightly better in the Southdowns.

More hay and silage were required per hundred pounds of single Hampshire lamb produced, on account of the much larger amounts eaten by the ewes, especially during the dry period; while more concentrates were required per hundred pounds of Southdown single lamb, on account of the longer nursing period of the ewes and growth period of the lambs.

The estimated total digestible nutrients required for each hundred pounds of final feed-lot lamb weight were practically the same for Southdown and Hampshire single lambs (table 2).

Slightly higher yield of dressed carcass for Southdown lambs gave them about a 3-percent advantage in total digestible nutrient requirement for each hundred pounds of dressed carcass as compared with the Hampshire single lambs.

A still higher yield of lean meat in the carcass gave the Southdown lambs a 13-percent advantage over the Hampshire singles in digestible nutrient requirement per hundred pounds of lean meat.

A Hampshire twin lamb's feed and its half of the mother's yearly feed was only slightly lower in concentrates but only half as high in alfalfa hay and silage as that of the Hampshire single lamb and its mother. Slightly less concentrates and about 20 percent less of the roughages were required than in the case of the Southdown single lamb and its mother.

The estimated total digestible nutrient content of the total feeds eaten per Hampshire ewe and single lamb was approximately 55 percent greater than that of the twin lamb and its half of the mother's feed. Nutrient requirement per hundred pounds of finished lamb weight was 50 percent greater for either the Hampshire or Southdown singles than for the Hampshire twins.

On the basis of the dressed carcass yield, Hampshire singles required 45 percent more total digestible nutrients than twins. On the basis of lean-meat yield, Hampshire singles required 62 percent more nutrients than twins, while Southdown singles required 41 percent more than the Hampshire twins.

On the basis of total digestible nutrient requirement per hundred pounds of lean meat, a Hampshire lamb crop of one pair of twins to each four single lambs, or a 120-percent lamb crop, would be necessary to equal a Southdown lamb crop of all single lambs. A summary of unpublished data from the University of Illinois sheep flock over an 18-year period shows that for each 100 Hampshire ewes, 148 lambs were born; while for each 100 Southdown ewes, 122 lambs were born; or for each 100 Southdown lambs, 122 Hampshire lambs were born.

Hampshire single lambs born in February were ready for market in June at an average age of 117 days, while Hampshire twin lambs and Southdown single lambs born at the same time were not ready until middle or late July, at ages of 155 and 164 days, respectively. Average feed-lot weights were as follows: Southdown singles, 65.57 pounds; Hampshire singles, 85.93; and Hampshire twins, 84.43.

The average Southdown lamb was graded Top Choice both on foot and in carcass, while the average Hampshire was Low Choice in grade in both cases.

According to Chicago lamb quotations by grades for the years 1936, 1937, and 1938, as shown in table 3, the average price advantage of Low Choice Hampshire single lambs in June was about \$1 per hundred pounds as compared with Top Choice Southdown single lambs in late July or early August. Low Choice Hampshire twin lambs ready for market about the same time as Southdown single lambs were worth slightly less than the Southdown lambs because of lower grade; and they were worth fully \$1.08 per hundred pounds less than Hampshire single lambs because of lower prices in July than in June.

TABLE 3.—Chicago prices per hundred pounds of live lambs by grades, expressed as monthly averages for 1936-38

Month	Choice	Good	Medium	Plain
May.....	\$11.37	\$10.86	\$10.13	-----
June.....	11.26	10.74	9.89	\$8.44
July.....	10.18	9.63	8.68	7.50
August.....	9.65	9.48	8.52	7.27
September.....	9.64	9.16	8.13	6.86
October.....	9.23	8.82	7.85	6.60
November.....	9.33	8.91	7.96	6.68

The greater the proportion of twins among the Hampshires, the greater the saving in feed per hundred pounds of lamb, although a lower average selling price would be expected since fewer single lambs were ready for the early market.

Lambs born in March instead of February and growing at a similar rate would be ready for market a month later than those in this experiment. In such cases the price of Hampshire singles in July, on the basis of prices during the last 3 years, would be about 20 cents higher than that of Southdown singles in August, as compared with \$1 difference in price a month earlier.

Higher yield of boneless meat from each hundred pounds of live lamb made Southdown lambs worth \$11.13 when Hampshire lambs were worth \$10 per hundred pounds. This would be fully as great an advantage for Southdown lambs in a discriminating market as the price advantage for Hampshire lambs if sold on the June market.

The content of the digestive tract in the feed-lot was 2.73 percent more for Hampshire lambs than for Southdowns on the basis of empty body weight. Other offal and byproducts at slaughter were also higher for Hampshire lambs, resulting in 2 percent more warm carcass yield for Southdowns on the basis of empty weights, and 3.04 percent more chilled carcass on the basis of slaughter weight. Both of these differences were highly significant according to Fisher's method for small numbers.

Numerical differences in average percentages of retail cuts as proportions of the carcass were small, ranging from 0.03 to 0.61 percent, and were not statistically significant. Average total percentages of the four major cuts, leg, loin, rib, and shoulder, were 73.40 and 73.26 for Southdowns and Hampshires, respectively.

Average content of bone in the right half-carass was 3.28 percent higher numerically in the Hampshire carcasses than in the Southdowns, a highly significant difference. Bone content of each retail cut and bone trim in the carcass were significantly higher for Hampshire lambs. (Table 4).

TABLE 4. — Statistically significant differences in composition of carcasses and cuts from purebred lambs

Portion of carcass	Average composition		Difference in favor of Southdown	Standard error of difference	t value ¹	Significance
	Southdown	Hampshire				
Bone in right half of carcass	Percent 13.05	Percent 16.33	Percent -3.28	0.5638	5.818	High.
Bone in trimmed right-side cuts:						
Leg	12.96	16.53	-3.57	.4807	7.425	Do.
Loin	10.33	12.51	-2.18	.7405	2.95	Do.
Rib	16.94	20.13	-3.19	1.074	2.97	Do.
Shoulder	13.61	16.94	-3.33	.751	4.44	Do.
Breast	12.58	16.73	-4.15	.709	5.85	Do.
Shank	31.44	40.48	-9.04	1.333	6.78	Do.
Bone trim as proportion of carcass	1.51	1.89	-.38	.0697	5.452	Do.
Lean meat as proportion of trimmed right-side cuts:						
Leg	66.55	64.46	2.09	1.017	2.055	Significant.
Rib	52.67	49.15	3.52	1.619	2.17	Do.
Shank	49.99	46.44	3.55	1.131	3.14	High.
Selected lambs: ²						
Lean meat as proportion of:						
Right-half carcass	55.89	53.63	2.26	.936	2.41	Significant.
Trimmed right leg	67.20	65.33	1.87	.780	2.40	Do.
Fat as proportion of right shank	16.70	11.80	4.90	1.009	4.856	High.
Lean and fat as proportion of right half of carcass	80.50	76.79	3.71	.5386	6.888	Do.

¹ FISHER (5, table 11):

² 10 Southdown and 15 Hampshire lambs intermediate in fatness.

Yield of boneless meat was, conversely, higher for Southdown lambs, averaging 3.71 percent above that in Hampshire lambs—a highly significant difference.

Average content of mechanically separable lean was 1.9 percent higher in the half carcass of Southdown lambs, but the difference was

was not significant when all lambs were included. When extremely thin and fat carcasses were excluded, averages of 10 Southdowns and 15 Hampshires gave a difference of 2.26 percent, which is significant. Average content of lean as percent of the trimmed cut was significantly higher for Southdowns in the leg, rib, and shank only when all lambs are considered.

Average fat content separated with the knife was not significantly different in the half carcass or in any cut except the foreshank. Southdown half carcasses averaged 1.81 percent higher in fat, and they were higher in all cuts except carcass trim, loin, and rib. If fat trim were added to the other carcass fat, Southdowns would be only 1.2 percent higher in fat content than Hampshires.

Average area of eye muscle on the anterior surface of the loin cut as measured with a planimeter was not significantly different for 11 Southdown lambs and 12 Hampshire twin lambs measured.

Correlations were high between area of loin eye muscle and weights of each of the following parts among Hampshire lambs, or Hampshire and Southdown lambs together: Right half carcass, lean in the half carcass, loin eye muscle, loin lean, and rib eye muscle. Correlation of area of loin eye with weight of Southdown loin eye, and lean in the half carcass, and with Hampshire rib lean was significant in each case, but not highly so.

Color readings on the rib eye muscle by means of a spectrophotometer, giving wave lengths for hue in millimicrons, and percentages of brightness and purity, showed no differences between the two groups of lambs, but indicated a relatively bright pinkish red color as seen by the eye.

Dry substance and ether extract each averaged slightly higher in the boneless meat of Southdown carcasses than in that of Hampshire carcasses, but the differences were not statistically significant. When divided into content of lean and of fat separately, there were significant but small differences in total nitrogen of the lean, and in dry substance and ether extract of the fat, the difference favoring Southdowns in each case. Both physical and chemical analyses of the carcasses indicated a similar degree of finish in the Southdown and Hampshire lambs.

Apparent digestibility of a full ration of equal parts of corn and hay by six pairs of purebred Southdown and Hampshire yearling sheep was similar except for the total nitrogen, as shown in table 5. An average of 60.2 percent of the total nitrogen was digested by the Hampshires as compared with 55 percent by the Southdowns, a highly significant difference. Each Hampshire excelled its pair mate in the use of nitrogen and also in most cases in the use of other nutrients. Average coefficients of digestibility for dry matter, crude fiber, and gross energy were higher for the Hampshires, but differences were not significant. Feed consumption by three pairs of lambs when full-fed during a 3-week preliminary period was found to be on the basis of the three-fourths power function of the body weight. These results formed the basis of feed allotment and the other six lambs were paired satisfactorily on a similar basis.

TABLE 5.—*Summary of digestion coefficients for purebred lambs 12 months old, full-fed*

Breed, number, and sex of animals	Weight of sheep	Average daily feed	Digestion coefficient of—			
			Dry matter	Gross energy	Crude fiber	Total nitrogen
Southdowns:	<i>Pounds</i>	<i>Grams</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
A-1, ram	71	840	69.8	68.6	42.8	54.7
B-3, ewe	80	920	71.0	70.2	39.7	56.6
C-5, ewe	88	988	69.6	68.4	36.0	55.2
D-7, ewe	71	780	70.3	69.2	38.3	57.2
E-9, ewe	91	944	69.3	68.0	39.1	54.4
F-11, ewe	95	852	67.0	65.6	33.1	52.0
Average	82.7	887	69.5	68.3	38.2	55.0
Hampshires:						
A-2, ram	155	1,512	73.2	71.8	43.2	64.1
B-4, ewe	132	1,340	72.1	70.7	43.9	58.4
C-6, ewe	143	1,420	70.8	70.0	40.0	58.9
D-8, ewe	118	1,144	69.9	68.2	38.2	61.9
E-12, ewe	139	1,296	70.4	68.9	40.5	59.9
F-10, ewe	125	1,048	68.9	67.8	37.6	58.3
Average	135.3	1,293	70.9	69.6	40.6	60.2
Mean difference	52.6	406	1.38	1.23	2.4	5.23
One-half <i>P</i> by Fisher's method for small numbers.			.07	.125	.097	.005
Significance			None	None	None	High

There was no significant difference between Southdown-sired grade lambs and Hampshire-sired lambs from similar ewes in ability to digest a maintenance or slightly above maintenance ration of equal parts of grain and hay. These 20 pairs of lambs were fed on the basis of the 0.74 power of body weight, which is in line with suggestions by Kleiber (12) and by Brody, Proctor, and Ashworth (4). The Southdown-sired lambs averaged 79.5 and the Hampshire-sired lambs 69.5 pounds in weight, which is contrary to expectation in the case of purebreds of the two breeds. While the heavier lambs tended to be more efficient, the differences were not statistically significant.

TESTS WITH LAMBS FROM GRADE WESTERN EWES

In the Dixon Springs project, located in Pope and Johnson Counties in southeastern Illinois, a program supervised by the University of Illinois in cooperation with the United States Government is being developed in an effort to establish pasture grasses on the worn-out land typical of some of that in several States. A flock of 360 western ewes was available in this area for the production of lambs to be raised under southern Illinois conditions.

One group of 112 2-year-old ewes was apparently of predominant Merino or Rambouillet breeding and originated in Montana. Another group of 248 white-faced ewes originating in the State of Washington had the appearance of sheep sired by long-wooled rams and out of fine-wooled ewes. The crossbred ewes averaged 104 pounds in weight in September as yearlings, as compared with 92 pounds for the 2-year-old fine-wooled ewes.

METHOD OF PROCEDURE

The fine-wooled and crossbred ewes were in each case divided into three lots as nearly uniform as possible according to weight, conformation, fleshing, fleece, and other characteristics commonly considered in breeding ewes. Throughout the experiment all ewes were either fed and pastured together as one flock or else the rations and management were as nearly identical as possible. Rams of the three breeds—Hampshire, Shropshire, and Southdown—were placed with the respective lots of ewes on September 10, 1937. The three Hampshire rams were yearlings, weighing 158, 160, and 162 pounds; the Shropshires were also yearlings and weighed 135, 144, and 159 pounds; one of the three Southdowns was an aged ram weighing 160 pounds and the other two were yearlings weighing 118 and 132 pounds, respectively.

On July 25, 1938, 72 percent of all wether lambs were sold on the Chicago market. The remaining wethers were weaned shortly afterward and fed grain on pasture until marketed on October 17. The ewe lambs were weaned August 8, and were weighed and graded at the farm by a lamb salesman from a Chicago livestock commission firm. They were not marketed, but market weight was estimated from the shrink on the wether lambs.

Complete data concerning weights and live and carcass grades were obtained on the wether lambs on the market and in Armour's and Swift's packing houses. Each lamb carcass was graded by the supervisor of Federal meat grading for the Chicago district. Since all wether lamb carcasses were graded more carefully by the Federal supervisor than it was possible to do with the live lambs, carcass grades for the wethers were used in computing values. Only single lambs were used in the data analyzed.

Monthly market prices of live lambs by grades at Chicago, as reported by the Bureau of Agricultural Economics of the United States Department of Agriculture for the years 1936, 1937, and 1938, (shown in table 3) were used in assigning values. There was a sharp decline in price from June to July and a gradual fall until October, with a slight rise in November. There was a similar trend for each grade of lambs, resulting in a similar spread in prices between grades from month to month. Average value per lamb in each group was obtained from the sum of all lambs by grades. Those of each grade were valued at the price for the month when graded or marketed.

RESULTS

Time of marketing, weight, and grade were factors determining the value per lamb. Average birth dates of all groups of lambs, according to breeding or according to time of marketing, were similar. More Hampshire- and Southdown-sired wether lambs were ready for the higher market in July than of Shropshire-sired wethers, the percentages being 76, 79, and 61, respectively.

Distribution of lambs by farm weights is indicated in table 6 and by grades in table 7. The Hampshire-sired lambs were characterized by slightly higher weights, while Southdown lambs were outstandingly highest in grade. The distribution of lambs by weights and by grades was similar for those of each sex. The weights of wether lambs were slightly higher than those of ewe lambs on account of the marketing of

some wether lambs later after further feeding, but the results among lots were not materially changed thereby.

TABLE 6.—Distribution of lambs from grade western ewes by farm-weight classes

Description of lambs	Lambs in weight class—							Average weight of lambs in group
	90 or more pounds	85-89 pounds	80-84 pounds	75-79 pounds	70-74 pounds	65-69 pounds	64 pounds or less	
Wether lambs from—								
Crossbred ewes:	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Pounds</i>
Hampshire sire	3	5	7	4	8	4	—	78.74
Shropshire sire	—	2	9	5	5	2	3	75.15
Southdown sire	2	4	10	2	10	1	3	77.19
Fine-wooled ewes:								
Hampshire sire	1	3	3	3	—	—	—	83.0
Shropshire sire	4	—	3	7	4	2	3	76.78
Southdown sire	—	3	5	1	3	3	1	76.3
All ewes:								
Hampshire sire	4	8	10	7	8	4	—	79.8
Shropshire sire	4	2	12	12	9	4	6	75.9
Southdown sire	2	7	15	3	13	4	4	76.9
All rams:								
Crossbred ewes	5	11	26	11	23	7	6	77.18
Fine-wooled ewes	5	6	11	11	7	5	4	77.9
Ewe lambs from—								
Crossbred ewes:								
Hampshire sire	—	3	3	2	5	4	1	75.1
Shropshire sire	—	2	5	6	7	2	3	74.3
Southdown sire	—	1	4	4	6	1	4	73.5
Fine-wooled ewes:								
Hampshire sire	1	5	—	4	2	3	1	76.4
Shropshire sire	—	—	—	2	6	5	1	70.4
Southdown sire	—	—	—	4	5	5	—	71.1
All ewes:								
Hampshire sire	1	8	3	6	7	7	2	75.7
Shropshire sire	—	2	5	8	13	7	4	72.9
Southdown sire	—	1	4	8	11	6	4	72.5
All rams:								
Crossbred ewes	—	6	12	12	18	7	8	74.3
Fine-wooled ewes	1	5	—	10	13	13	2	72.8

TABLE 7. Distribution of lambs from western ewes by market grades

Market grades	Choice	Good	Medium	Plain	Cull	Total
Carcass grades of wether lambs from—	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>
Crossbred ewes:						
Hampshire sire	1	18	7	5	—	31
Shropshire sire	1	17	5	3	—	26
Southdown sire	22	9	1	—	—	32
Fine-wooled ewes:						
Hampshire sire	1	7	1	1	—	10
Shropshire sire	—	11	11	—	1	23
Southdown sire	6	10	—	—	—	16
All ewes:						
Hampshire sire	2	25	8	6	—	41
Shropshire sire	1	28	16	3	1	49
Southdown sire	28	19	1	—	—	48
All rams:						
Crossbred ewes	24	44	13	8	—	89
Fine-wooled ewes	7	28	12	1	1	49
Live grades of ewe lambs from—						
Crossbred ewes:						
Hampshire sire	—	5	9	2	2	18
Shropshire sire	—	14	5	—	1	25
Southdown sire	6	10	3	1	—	20
Fine-wooled ewes:						
Hampshire sire	2	8	3	2	1	16
Shropshire sire	2	8	4	—	—	14
Southdown sire	3	9	1	1	—	14
All ewes:						
Hampshire sire	2	13	12	4	3	34
Shropshire sire	7	22	9	—	1	39
Southdown sire	9	19	4	2	—	34
All rams:						
Crossbred ewes	11	29	17	3	3	63
Fine-wooled ewes	7	25	8	3	1	44

TABLE 8.—Market weights and value per animal of lambs from grade western ewes

Wether and ewe lambs	Lambs	Average weight per lamb	Average value per lamb	Wether and ewe lambs	Lambs	Average weight per lamb	Average value per lamb
From crossbred ewes:	<i>Number</i>	<i>Pounds</i>	<i>Dollars</i>	From all ewes:	<i>Number</i>	<i>Pounds</i>	<i>Dollars</i>
Hampshire sire	49	73.06	6.37	Hampshire sire	75	73.56	6.52
Shropshire sire	51	70.53	6.46	Shropshire sire	88	70.39	6.38
Southdown sire	52	71.62	6.88	Southdown sire	82	70.93	6.80
From fine-wooled ewes:				By all rams:			
Hampshire sire	26	74.50	6.79	Crossbred ewes	152	71.72	6.58
Shropshire sire	37	70.10	6.27	Fine-wooled ewes	93	71.25	6.54
Southdown sire	30	69.73	6.66				

The records for all lambs are combined in table 8 to show average market weight and value per lamb by lots. Less spread in live grades and values among ewe lambs than among wether lambs, which were graded in the carcass, tended to narrow the spread in value among all lambs. The highest value per ewe lamb was \$6.53 and the lowest value \$6, as compared with \$7.28 and \$6.34 for wether lambs. The maximum value for all lambs was \$6.88 and the minimum value \$6.27.

The averages of all lambs showed no appreciable difference between lambs from fine-wooled ewes weighing 92 pounds and those from crossbred ewes weighing 104 pounds.

Concerning the influence of the sire, the data show that Southdown-sired lambs averaged 28 cents more in value per lamb, although 2.63 pounds lighter in weight than Hampshire-sired lambs. Shropshire-sired lambs were 3.17 pounds lighter and 14 cents less valuable than Hampshire-sired lambs.

The heavy weight of both wether and ewe lambs sired by Hampshire rams and out of fine-wooled ewes was an advantage, but a correspondingly high value per lamb was not realized on account of lack of fatness.

Rank according to value per lamb of all lots of lambs differing in sire or dam was as follows: Southdown \times crossbred, Hampshire \times fine wool, Southdown \times fine wool, Shropshire \times crossbred, Hampshire \times crossbred, and Shropshire \times fine wool.

The influence of the sire, regardless of the dam, resulted in the following rank according to value per lamb: Southdown, Hampshire, and Shropshire.

CONCLUSIONS AND SUMMARY

In view of the data obtained in comparing Hampshire and Southdown breeding ewes, it seems reasonable to conclude that mutton-type ewes of different breeds differing markedly in body weight consume feed approximately in proportion to body weight when grazing on good pasture, or when full-fed appropriate rations for normal functions. On the other hand, lambs fed a maintenance ration, or those full-fed for rapid growth and fattening, consume feed on the basis of approximately the three-fourths power function of body weight.

Single lambs of Hampshire and Southdown breeds require total digestible nutrients, including yearly feed for the ewes, in approximately equal quantities per unit weight of Choice grade market lamb. Comparatively larger quantities of feed during the longer dry period are required by Hampshire ewes, whereas larger total quantities over

a longer time are required by the Southdown nursing ewes and growing lambs. However it seems possible that under usual farm practices, early Hampshire lambs being pushed for the June market may require relatively larger quantities of grain before the pasture season, whereas Southdown lambs may make relatively greater use of spring and summer pasture.

A twin lamb of the large early-maturing Hampshire breed requires total digestible nutrients, including its half of the ewe's yearly feed, in approximately two-thirds the quantity necessary for a similar Choice grade single lamb, or for a similar market weight of Choice grade single lamb of the small Southdown breed.

Southdown lambs of Choice market finish give higher yields of dressed carcass, boneless meat, and separable lean meat than do Hampshire lambs similar in finish; and thus are worth a higher price as live lambs, in the carcass, and in the retail trade.

Approximately a 120-percent Hampshire lamb crop, or one pair of twins to four single lambs, is necessary to produce lean meat from a carcass of Choice finish as efficiently, with respect to total digestible nutrients, as a crop of Southdown single lambs.

It may be assumed that some twins among Southdowns will tend to reduce feed costs of production, as was true among Hampshires.

Well-fed Hampshire single lambs attain Choice market finish approximately 6 weeks younger than Southdown single lambs or Hampshire twin lambs.

Southdown live lambs on a discriminating market should have as great an advantage in price per pound owing to the higher yield of boneless meat as Hampshire lambs that mature early in the season when prices are higher, and a greater advantage than late-marketed Hampshire lambs.

Area of loin eye muscle is a fairly good index of the lean meat content of the lamb carcass.

The color of lean is similar in Southdown and Hampshire Choice lamb carcasses.

The content of dry matter, total nitrogen, and ether extract is similar in boneless meat of Hampshire and Southdown lambs of Choice market grade.

The efficiency of digestion in grade lambs sired by large and small breeds, and fed on maintenance or slightly above maintenance rations, is unaffected by the breed of the sire or by the weight of the lamb. The data on purebred lambs of large and small breeds, when on full feed and about 1 year of age, indicate greater efficiency in the digestion of protein by those of the larger breed.

Market finish is a major factor in determining grade, and is important along with market weight and time of marketing in determining the value of a lamb.

There is little difference between fine-wooled and crossbred type western white-faced ewes such as were used in this experiment, either in weight or value of lamb produced when the ewes were mated with mutton-type rams. If feed consumption by the ewes is approximately in proportion to body weight, as was found to be true in comparing purebred Hampshire and Southdown ewes, the heavier crossbred ewes may be less efficient in respect to feed consumption than the fine-wooled ewes.

The small breed of sheep, Southdowns, compares favorably with the large breed of sheep, Hampshires, for economical production of market lambs in purebred flocks of the two breeds; while the use of Southdown rams with western ewes seems to be fully as desirable as the use of Hampshire rams under Central States conditions and with Federal grading of the carcasses.

Inheritance of size by the offspring is more obviously different between Hampshire and Southdown rams when they are mated with purebred ewes of the respective breeds than when they are mated with grade western ewes.

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EFFECT OF ALGAE IN RELATION TO AERATION, LIGHT, AND SOURCES OF PHOSPHORUS ON GROWTH OF TOBACCO IN SOLUTION CULTURES¹

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INTRODUCTION

It is generally recommended that light be excluded from the roots of plants grown in solution cultures, the assumption being that this prevents the development of algae that compete with the plant. It has been shown that tobacco (*Nicotiana tabacum* L.) plants grown in solution cultures give a decided response to aeration when the solution is bubbled with compressed air.²

There are very few references in the literature to the effects of light on the root growth of plants in solution cultures, and only one has come to the attention of the writers relating to what is usually a concomitant effect of the admission of light to the roots, namely, the growth of green algae in the solution. Knight,³ studying the response of plants in water cultures to aeration of the roots, used sprigs of the aquatic herb *Elodea canadensis* Michx. as one method of aeration. He found that aeration by *Elodea* had a beneficial effect on the growth of maize, but that this effect was soon obscured by the growth of algae in the solution, which appeared to do more effectively what *Elodea* had done. There was increased root growth in solutions in which algae had multiplied. No weights of roots were taken, but it was concluded from examination that the increase in growth was much too large to be accounted for by the algae alone.

The results of the preliminary experiment described below led the writers to attempt to determine quantitatively the differences that might be expected and other effects that might be observed. Three experiments were made, the results of which are reported herein.

MATERIAL AND METHODS

The stock solutions, which were diluted 20 times for growing plants, are shown in table 1. The experimental procedures were in general those described in previous publications.^{4 5} Any differences in treatment employed will be mentioned in connection with the presentation of results.

¹ Received for publication January 22, 1940.

² McMURTREY, J. E., JR. THE EFFECT OF BORON DEFICIENCY ON THE GROWTH OF TOBACCO PLANTS IN AERATED AND UNAERATED SOLUTIONS. *Jour. Agr. Res.* 38: 371-380, illus. 1929.

³ KNIGHT, R. C. THE RESPONSE OF PLANTS IN SOIL- AND IN WATER-CULTURE TO AERATION OF THE ROOTS. *Ann. Bot. [London]* 38: 305-325, illus. 1924.

⁴ See footnote 2.

⁵ McMURTREY, J. E., JR. DISTINCTIVE EFFECTS OF THE DEFICIENCY OF CERTAIN ESSENTIAL ELEMENTS ON THE GROWTH OF TOBACCO PLANTS IN SOLUTION CULTURES. U. S. Dept. Agr. Tech. Bul. 340, 43 pp., illus. 1933.

TABLE 1.—*Volume-molecular concentrations of chemically pure salts used in preparing stock solutions*

Solution		Volume-molecular concentration of—					
Name	Designation	Ca(NO ₃) ₂	CaH ₄ (PO ₄) ₂	CaHPO ₄	Ca ₃ (PO ₄) ₂	CaCO ₃	KNO ₃
Monopotassium phosphate.....	C ₁	0.1235	0.0217
Monocalcium phosphate.....	P ₁	.1020	0.02110637
Dicalcium phosphate.....	P ₂	.0813	0.04220637
Tricalcium phosphate.....	P ₃	.1024	0.02110637
Calcium carbonate and ammonium nitrate.....	C ₂0052	0.1180
Calcium carbonate and no added nitrogen.....	N00521180

Solution		Volume-molecular concentration of—						
Name	Designation	KH ₂ PO ₄	Mg(NO ₃) ₂	MgSO ₄	NH ₄ NO ₃	NH ₄ Cl	K ₂ HPO ₄	MgCl ₂
Monopotassium phosphate.....	C ₁	0.0422	0.0124	0.0125	0.0280
Monocalcium phosphate.....	P ₁0125	0.0266	0.0141
Dicalcium phosphate.....	P ₂0124	.0125	.0170	.0280
Tricalcium phosphate.....	P ₃0125	.02660141
Calcium carbonate and ammonium nitrate.....	C ₂0125	.1600	0.0320	.0141
Calcium carbonate and no added nitrogen.....	N01250320	.0141

EXPERIMENTAL DATA

PRELIMINARY EXPERIMENT

In the spring of 1937, 20 tobacco plants of the Connecticut Broadleaf variety were put into twenty 2-quart glass fruit jars containing a complete nutrient solution (C₁). Ten of these jars were covered with several thicknesses of manila paper, while 10 had no covering of any kind and were exposed to full sunlight. In a week or 10 days it was observed that the plants in the uncovered jars were making new root growth and new top growth much more rapidly than those in the covered jars. The uncovered jars all had a thin film of algae on their sides and bottoms in addition to many algae floating throughout the solution.

Later it was observed that the roots of the plants in the uncovered jars grew down to the bottom of the jars, while those in the covered jars grew short and were bunched near the surface of the solution.

After growing about a month longer, the roots and tops were measured and the plants were harvested and air-dried. Total measurements and weights are shown in table 2.

TABLE 2.—*Average measurements and air-dry weights of tobacco plants grown in nutrient solution in covered and uncovered containers*

Condition of containers	Plants	Length of roots	Height of plant	Air-dry weight		
				Tops	Roots	Total
Covered.....	Number 10	Centi-meters 5	Centi-meters 47	Grams 10.92	Grams 1.81	Grams 12.73
Uncovered.....	10	31	69	20.85	2.45	23.30

TESTS IN COVERED AND UNCOVERED ROOT CONTAINERS WITH THREE CALCIUM SALTS OF PHOSPHORUS

Since the results of the preceding experiment were rather striking, it was decided to carry out a greatly extended and controlled experiment and use three different nutrient solutions. There were 40 plants in each solution. One-half of the plants in each solution had been previously disinfected by treatment with silver nitrate (AgNO_3), 1 to 1,000; 1 group of each of the 2 lots was aerated artificially by means of compressed air; one-half of the plants of each of the four groups thus formed were put into uncovered 2-quart glass fruit jars, and the other half into covered jars. These were so arranged that all possible combinations were obtained, thus:

Monocalcium phosphate nutrient solution (P_1)	{ Covered	Unaerated	{ Roots not disinfected Roots disinfected
		Aerated	{ Roots not disinfected Roots disinfected
	{ Uncovered	Unaerated	{ Roots not disinfected Roots disinfected
		Aerated	{ Roots not disinfected Roots disinfected

Hence, there were 8 combinations of 5 plants each in each of the 3 nutrient solutions. All nutrient solutions were the same except for the source of phosphorus, which in the second group was supplied as dicalcium phosphate (P_2) and in the third group as tricalcium phosphate (P_3).

Before the plants were placed in the experimental solutions, they were allowed a start of about 2 weeks in dicalcium phosphate solution in uncovered, unaerated jars. At the time of transfer to the new solution (October 5) the plants not disinfected had made considerably more root and top growth than those disinfected with silver nitrate. All roots were rinsed with distilled water before the plants were placed in the new solution. Each culture was inoculated with 10 cc. of a suspension of green algae. Boron, manganese, and iron (as ferric citrate) were added at this time.

On October 18 and again on October 28 all solutions were completely renewed. On November 4, 100 cc. of stock nutrient solution was added to each culture, and on November 12 all solutions were completely renewed for the last time. The plants were harvested on November 22.

The following algae⁶ were found: *Protococcus viridis* var. *infusionum* Rab., *P. viridis* var. *angulosa* Menegh., *Ulothrix parietina* (Vauch.) Kg., *Euglena viridis* Ehrb., *Chroococcus turgidis* Naeg., and *Aphanocapsa virescens* Rab. *P. viridis angulosa* occurred in greatest abundance. These are all unicellular green algae with the exception of *U. parietina*, a filamentous green alga that Dr. Chase states is often found growing with *Protococcus*. Those examined from the monocalcium phosphate cultures were greener and the cells were larger than those from cultures supplied phosphorus as dicalcium phosphate. The algae from cultures that were supplied with phosphorus from tricalcium phosphate were not examined.

⁶ An examination of the algae growing in these solutions was made by Dr. Florence Meier Chase, of the Division of Radiation and Organisms, Smithsonian Institution.

In table 3 are shown the average measurements, oven-dry weights, and percentage of leaf, stalk, and root of the plants grown in nutrient solutions P₁, P₂, and P₃ respectively. Since the differences between the various treatments in solutions P₁ and P₂ are nearly parallel, the data from solution P₂ will be discussed as representative.

TABLE 3.—Average measurements, oven-dry weights, and percentages of leaf, stalk, and roots of tobacco plants grown in various nutrient solutions

MONOCALCIUM PHOSPHATE (P₁)

Treatment	Height	Length of roots	Oven-dry weight				Distribution of oven-dry weight		
			Leaf	Stalk	Root	Total	Leaf	Stalk	Root
	<i>Centimeters</i>	<i>Centimeters</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Roots disinfected:									
Uncovered, aerated	94	58	15.76	9.19	2.90	27.85	56.6	33.0	10.4
Covered, aerated	109	50	21.05	14.08	3.81	38.94	54.1	36.2	9.8
Uncovered, unaerated	88	37	12.21	9.88	3.36	25.45	47.9	38.8	13.2
Covered, unaerated	64	31	5.96	4.62	1.38	11.97	49.8	38.6	11.6
Roots not disinfected:									
Uncovered, aerated	118	46	17.81	15.83	4.84	38.49	46.3	41.1	12.6
Covered, aerated	135	37	20.31	19.11	5.1	44.56	45.5	42.9	11.5
Uncovered, unaerated	114	32	13.82	13.42	4.72	31.96	43.3	41.9	14.8
Covered, unaerated	91	28	10.08	8.39	3.12	21.59	46.7	38.9	14.4

DICALCIUM PHOSPHATE (P₂)

Roots disinfected:									
Uncovered, aerated	104	55	15.92	11.22	3.98	31.12	51.1	36.1	12.8
Covered, aerated	121	47	20.25	15.96	4.36	40.57	49.9	39.3	10.7
Uncovered, unaerated	95	32	12.87	9.54	3.66	26.07	49.4	36.6	14.0
Covered, unaerated	39	27	3.37	1.68	.73	5.78	58.3	29.1	12.6
Roots not disinfected:									
Uncovered, aerated	125	45	20.29	16.0	5.76	42.05	48.2	38.1	13.7
Covered, aerated	119	35	19.01	18.62	4.59	42.22	45.0	44.1	10.9
Uncovered, unaerated	105	28	12.96	12.0	4.25	29.21	44.4	41.1	14.5
Covered, unaerated	81	27	8.36	9.28	2.65	20.29	41.2	45.7	13.1

TRICALCIUM PHOSPHATE (P₃)

Roots disinfected:									
Uncovered, aerated	90	59	15.39	10.0	4.83	30.22	50.9	33.1	16.0
Covered, aerated	111	53	15.86	16.46	5.38	37.70	42.1	43.6	14.3
Uncovered, unaerated	48	44	8.41	5.21	3.12	16.74	50.2	31.2	18.6
Covered, unaerated	53	29	5.56	4.12	1.28	10.96	50.8	37.6	11.6
Roots not disinfected:									
Uncovered, aerated	114	49	17.74	16.50	7.20	41.44	42.8	39.8	17.4
Covered, aerated	128	37	17.20	19.65	5.92	42.77	40.2	46.0	13.8
Uncovered, unaerated	87	31	11.49	9.85	4.75	26.09	44.0	37.8	18.2
Covered, unaerated	67	28	6.44	6.62	2.24	15.30	42.1	43.3	14.6

Examination of the data shows that the most outstanding difference lay between the aerated and unaerated plants in covered jars. In the series in which the roots were disinfected the average weights were 40.57 and 5.78 gm., or a ratio of 7 to 1. In the corresponding series in which the roots were not disinfected, the average weights were 42.22 and 20.29 gm., or a ratio of 2.1 to 1. The smaller difference in the latter case is possibly due to the fact that the roots that were not disinfected made considerably more growth before being placed in the experimental solutions, so that the initial effect was sufficient to allow these plants to make fair growth even after being placed in unaerated solutions.

The difference between the plants that were disinfected and those that were not disinfected in the covered and aerated cultures, i. e.,

between 40.57 and 42.22 gm., respectively, while not great, probably also indicates the effect of this initial advantage. This advantage, however, was much greater at the start than at the end of the experiment, and might have been reversed had the plants been allowed to reach full maturity before harvesting. The same advantage was evident in the monocalcium and tricalcium phosphate solutions. That most of this gain was in stalk rather than leaf is shown by the relative percentages of these parts to total dry weight. In plants not disinfected the stalk represents 44 percent of the total weight as against 39 percent for disinfected plants, while the leaves of the plants that were not disinfected comprised 45 percent of the total weight as against 50 percent for the disinfected plants. The relatively greater proportion of stalk in plants not disinfected was apparently due to the well-developed and ripening seed capsules, which in disinfected plants were just forming at the time of harvesting.

Examination of the data for the uncovered cultures, and hence those in which green algae made abundant growth, shows the results of the small preliminary experiment to be further confirmed. The average weight of the plants in uncovered, unacrated jars was 26.07 gm., and that of the covered, unacrated cultures was 5.78 gm., a ratio of 4.5 to 1. Here the difference, while not as pronounced as that between covered aerated and covered unacrated plants, is still striking, and shows the effect of the algae in aerating the solution by the release of minute bubbles of oxygen. In full sunlight these bubbles were observed rising rapidly.

Taken as a whole, the roots of the plants in uncovered jars averaged a higher percentage of the total dry weight than those in covered jars, and this difference was apparently due to the weight of the algae on the roots. The actual difference of weight due to algae could not, of course, be determined.

A comparison of the plants in covered and uncovered jars, where both were aerated with compressed air, shows differences in favor of the covered cultures, though not nearly as great as the differences shown above. The plants with roots disinfected (fig. 1) show a ratio of 1.3 to 1 in favor of the covered jars, while there was practically no difference between the two groups with roots not disinfected. Here again, however, the percentage of leaf weight to total weight was greater in the case of disinfected plants, averaging 50 percent as against 42.8 percent for plants not disinfected. A greater percentage of stalk for the plants that were not disinfected again indicates a greater degree of maturity, with more weight in the maturing seed head.

One rather striking and unexpected result of the experiment was the relatively good growth made by the plants where tricalcium phosphate was used as the source of phosphorus (table 3). It is known that this form is almost insoluble in water, and in previous water-culture experiments the results with it were poor. Nevertheless, in this experiment the 10 plants in covered and aerated solutions (fig. 2) had an average dry weight of 40.24 gm., or practically the same as those in the dicalcium phosphate solution; while the 10 in uncovered aerated solutions averaged 35.83 gm. Of the latter, the untreated half averaged 41.44 gm., which is among the highest weights attained. Apparently the roots of these untreated plants had made enough growth before being placed in the experimental solutions to come into direct contact with the tricalcium phosphate

on the bottom of the jars, and were thus able to take up soluble phosphorus.

The unacrated plants (fig. 3) made much less growth than the aerated, but here again, as in the case of dicalcium phosphate, the plants in uncovered jars made much better growth than those in covered jars. In the case of the disinfected plants, those in uncovered solutions averaged 16.74 gm. and those in covered jars averaged 10.96 gm. Where the roots were not disinfected, the plants in uncovered jars averaged 26.09 gm. and those in covered jars averaged 15.30 gm. This advantage in favor of the plants that were not disinfected is greater than in the case of either monocalcium or dicalcium phosphate and is believed to be due to the same cause mentioned previously; namely that the greater initial length of the roots that were



FIGURE 1.—Tobacco plants placed in P_i culture solution October 5, 1937; roots disinfected with silver nitrate 1 to 1,000 dilution (photographed November 6, 1937; scale shown in inches): A, solution darkened and aerated; B, light admitted to solution, which was aerated; C, solution darkened and unaerated; D, light admitted to unaerated solution.

not disinfected at the time of transfer to the new solutions brought them into direct contact with the tricalcium phosphate almost from the start.

Further study of the data for tricalcium phosphate shows that the 10 covered and aerated cultures made much better growth than the 10 uncovered and unaerated, these weights averaging 40.24 and 21.41 gm., respectively. This might indicate that the natural aeration of the nutrient solution by algae is not, of itself, sufficient for the needs of the plant. However, it was observed that in the solutions aerated through the medium of algae alone the growth of algae on the roots and on the sides of the jars was very much greater than in those cultures which, in addition to aeration by algae, were also aerated artificially. It is possible that this check upon the growth of the algae by artificial aeration was due to the mechanical agitation of the solution; it is also possible that it was due to a changed chemical balance within the solution caused by artificial aeration. The same phenomenon was observed in the monocalcium and dicalcium phosphate solutions, but it was not so pronounced. At any rate, it



FIGURE 2. Tobacco plants placed in culture solution October 5, 1937; roots not disinfected (photographed November 6, 1937; scale shown in inches). Light admitted to unacrated culture solutions, with phosphorus derived from different sources: A, Monocalcium phosphate (P_1); B, dicalcium phosphate (P_2); C, tricalcium phosphate (P_3).



FIGURE 3.—Tobacco plants placed in culture solution October 5, 1937 (photographed November 6, 1937; scale shown in inches). Culture solutions darkened, with phosphorus derived from different sources: A, Monocalcium phosphate (P_1); B, dicalcium phosphate (P_2); C, tricalcium phosphate (P_3).

is apparent that up to a certain point aeration by algae is definitely beneficial to the plant; but when the algae, for whatever reason, become too abundant, actual competition for the available nutrients robs the growing plant. It is also probable that too great an accumu-

lation of algae upon the roots may result in partly stopping the intake of nutrients.

If the average weights of the plants in covered and uncovered cultures are observed as a whole, regardless of the other treatments, they compare as shown in table 4. The uncovered cultures are thus shown to have made better growth in all three solutions, on the whole, than the covered cultures. This difference was greatest in the case of dicalcium phosphate, amounting to about 18 percent. The uncovered monocalcium solutions showed an increase of 5.7 percent, and the tricalcium solutions 7.3 percent.

TABLE 4.—Average dry weight of tobacco plants as affected by the various treatments considered separately

Treatment	Plants in culture solution	Monocalcium phosphate (P ₁)	Dicalcium phosphate (P ₂)	Tricalcium phosphate (P ₃)
	<i>Number</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
Covered	20	29.27	27.21	26.68
Uncovered	20	30.94	32.11	28.62
Aerated	20	37.46	38.90	38.03
Unaerated	20	22.75	20.34	17.27
Aerated by algae	10	28.71	27.64	21.41
Unaerated	10	16.78	13.04	13.12

The advantage of aerated over unaerated solutions, regarded as a whole, is shown by the total weights in table 4.

Here the increase due to aeration is very great, amounting to 64.7 percent for monocalcium, 91.7 percent for dicalcium, and 120.2 percent for tricalcium phosphate solutions. The very great increase in growth with aeration in the case of tricalcium phosphate emphasizes again its effect in causing the roots to grow down into direct contact with this compound.

It may be argued that distinction should be made between the cultures that were aerated both naturally and artificially (i. e., by algae and compressed air together) and those that were aerated by algae alone. Such comparison between the cultures aerated by algae alone and those not aerated at all is shown in table 4. Here the increase due to algae alone over unaerated cultures was 71.1 percent for monocalcium, 111.9 percent for dicalcium, and 63.2 percent for tricalcium phosphate. The average increase in this case was 82.1 percent, as compared with an average increase of 92.2 percent in the aerated cultures.

REACTION OF NUTRIENT SOLUTIONS⁷

In order to determine to what extent the pH value of the nutrient solutions was changed during the course of the experiment, representative samples of the solutions were taken at the time of harvesting. These were tested by the quinhydrone electrode method. To obtain a basis for comparison, freshly made solutions of each of the three basic nutrient solutions were tested at the same time. The pH readings of the various solutions are shown in table 5.

⁷ Determinations of reaction were performed by G. E. Halliday, assistant biochemist, Division of Tobacco and Plant Nutrition.

TABLE 5.—*pH values of nutrient solutions when freshly made and at time of harvesting*

Solution and treatment	Monocalcium phosphate (P ₁)	Dicalcium phosphate (P ₂)	Tricalcium phosphate (P ₃)
	<i>pH</i>	<i>pH</i>	<i>pH</i>
Freshly made solution.....	4.2+	4.9	4.9+
Solution at time of harvesting:			
Roots disinfected:			
Uncovered, aerated.....	5.8	5.9	6.7
Covered, aerated.....	5.6	6.1	6.5
Uncovered, unaerated.....	6.8	6.9	6.8
Covered, unaerated.....	5.9	6.2	6.5
Roots not disinfected:			
Uncovered, aerated.....	6.1	6.1	7.0
Covered, aerated.....	6.0	6.2	6.7
Uncovered, unaerated.....	6.8	6.7	6.6
Covered, unaerated.....	6.4	7.0	6.9

It will be observed that the pH values of the freshly made nutrient solutions tend to approach what is generally considered the optimum for tobacco.⁸ These low pH values were temporary, however, for the readings taken at the time of harvesting all show a marked rise in their pH value.

In order to learn whether there was any correlation between the growth of the algae and the hydrogen-ion concentration of the solutions, the covered and uncovered jars were compared in pairs through each series. That is, an uncovered aerated solution was compared with a covered aerated solution, and an uncovered, unaerated solution was compared with a covered, unaerated solution. By this method of comparison, the uncovered solutions, i. e., those containing algae, in monocalcium phosphate were shown to have a higher pH value throughout. However, in the case of dicalcium phosphate, only one out of four pairs showed a higher pH value for the uncovered cultures, while in the case of tricalcium phosphate, three out of four showed a higher pH value in uncovered jars. The cultures were then compared, within each kind of solution, to learn whether there was any correlation between the hydrogen-ion concentration and artificial aeration. By this comparison, it can be seen that every aerated culture had a lower pH value than its corresponding unaerated culture, except in one case when they were the same: The uncovered aerated cultures in the disinfected group with tricalcium phosphate had a pH value of 7.0, the highest attained, as compared with a pH value of 6.6 for the corresponding unaerated cultures. Further examination (table 3) shows that these aerated cultures had a greater dry weight than their corresponding unaerated cultures.

Apparently, then, there is little correlation between the growth of algae and the hydrogen-ion concentration of the solution, and no close correlation between the growth of the tobacco plant and the hydrogen-ion concentration of the solution. The fact that none of the pH readings in this experiment was equal to or lower than that of the freshly made nutrient solution may have been due to the continuous presence of enough calcium ions to keep the pH value fairly high.

⁸ ANDERSON, P. J., OSMUN, A. VINCENT, and DORAN, W. L. SOIL REACTION AND BLACK ROOT ROT OF TOBACCO. Mass. Agr. Expt. Sta. Bul. 229, pp. 118-136, illus. 1926.

During the latter part of the experiment it was observed that some of the roots showed root rot. This is assuredly not new in experiments with water cultures, but the interesting feature was that all of these plants, or practically all, were in covered jars. At the time of harvesting, notes were taken on these roots and their number tabulated as to treatment (table 6).

TABLE 6.—*Number of tobacco plants with defective roots, with their respective treatments*

Treatment	Plants with defective roots	Reaction of solution
	Number	pH
Monocalcium phosphate; covered, aerated, not disinfected	1	6.0
Dicalcium phosphate; covered, aerated, not disinfected	2	6.2
Dicalcium phosphate; covered, unaerated, not disinfected	2	7.0
Tricalcium phosphate; covered, aerated, disinfected	1	6.5
Tricalcium phosphate; covered, aerated, not disinfected	1	6.7
Tricalcium phosphate; uncovered, unaerated, disinfected	1	6.8

It will be noted at once that, of the eight plants with defective roots, seven were in covered jars. It is true that six of the eight had not been disinfected with silver nitrate, thus increasing the chances for infection. Nevertheless, the fact that only one plant of those growing in uncovered jars showed infection, as compared with seven in the covered jars, appears to indicate a definite protective effect on the part of the light or the algae growing on the roots, or possibly both. Whether this was purely mechanical and due to the covering of algae on the roots, or partly mechanical and partly chemical could not be determined. It is possible, however, that the effect was partly chemical, for the roots of the great majority of the plants in uncovered jars were not completely covered with algae. These roots or parts of roots that to the unaided eye showed no film of algae were a pure, shining pearl white, in contrast to many of those in covered cultures, which, especially as they approached maturity, assumed a dull, yellowish tinge. Some of the algae-covered rootlets were examined under the microscope in order to learn whether there was any symbiotic relationship such as exists between mycorrhiza and the roots of certain trees. No such relationship was evident.

TESTS WITH BLUE-GREEN ALGAE

In February 1938 a further experiment was begun, the object of which was twofold: (1) To learn whether the results of the preceding experiment would be verified; and (2) to find whether certain species of blue-green algae could, in the absence of nitrogen, fix enough atmospheric nitrogen in the nutrient solution to appreciably affect the growth of tobacco plants. Two nutrient solutions were used, one with nitrogen added (C_2) and the other with nitrogen withheld (N).

The cultures were arranged in groups of five plants each, as in the preceding experiment. Five cultures inoculated with blue-green algae were matched with five under similar conditions of exposure to light and aeration but inoculated with green algae. Of the latter, three

in each group were inoculated with 10 cc. each of an infusion of unicellular algae, and two with 10 cc. each of an infusion of filamentous algae.

The species of blue-green algae used was *Nostoc muscorum* Ag.,⁹ which is capable of more rapid nitrogen fixation than other known species of blue-green algae.¹⁰ For that reason it was chosen for this experiment. Allison found that this species was able to fix 18 to 30 mg. of nitrogen per 100 cc. of solution when a small amount of glucose was added. Its nitrogen-fixing ability is greatly increased when glucose is added. However, glucose was not used in this experiment.

On February 6 the experiment was set up. All jars, stoppers, glass tubes, and rubber hose were steam-sterilized. One hundred and twenty plants of the Connecticut Broadleaf variety, carefully selected for uniformity, were washed and then disinfected with silver nitrate, 1 to 1,000, the roots for 5 minutes and the tops for 2 minutes. Each nutrient solution was arranged as shown below:

Blue-green algae.....	{ Covered.....	{ Aerated
		{ Un-aerated
	{ Uncovered.....	{ Aerated
		{ Un-aerated
Green algae.....	{ Covered.....	{ Aerated
		{ Un-aerated
	{ Uncovered.....	{ Aerated
		{ Un-aerated
Control (no algae).....	{ Covered.....	{ Aerated
		{ Un-aerated
	{ Uncovered.....	{ Aerated
		{ Un-aerated

The culture of *Nostoc* as received was in the form of large, thin, but somewhat tenacious films. In order to supply these to the nutrient solutions in as nearly equal quantities as possible, the *Nostoc* was well shaken up with sterilized glass beads. This procedure broke up the films very satisfactorily. Each culture to receive *Nostoc* was then inoculated with 10 cc. of this infusion. Iron and manganese were added to all cultures, and on April 2, 2 cc. of zinc sulfate solution was added to each culture, as several were beginning to show signs of what appeared to be zinc deficiency. On April 11 all of the nitrogen cultures were completely renewed. Measurements of height and length of roots were taken at biweekly intervals throughout the growing period. On May 11 the plants were harvested and hung for air-drying.

The measurements and air-dry weights of the plants in cultures to which ammonium nitrate was added are shown in table 7.

⁹ Supplied as a pure culture by F. E. Allison, of the Fertilizer Research Division, Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture.

¹⁰ ALLISON, FRANKLIN E., HOOVER, SAM R., and MORRIS, HERMAN J. PHYSIOLOGICAL STUDIES WITH THE NITROGEN-FIXING ALGA, *NOSTOC MUSCORUM*. Bot. Gaz. 98: 433-463, illus. 1937.

TABLE 7.—Average measurements during growth period and air-dry weights of tobacco plants grown in nutrient solution with added nitrogen, with and without green algae and the blue-green alga *Nostoc muscorum*

Treatment	Average height of plants					Average lengths of roots					Average air-dry weight per plant			
	Mar. 4	Mar. 18	Mar. 31	Apr. 15	Apr. 29	Mar. 4	Mar. 18	Mar. 31	Apr. 15	Apr. 29	Roots	Leaves	Stalks	Total
Covered:	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>
G, ¹ unaerated	5	6	9	19	33	11	12	13	14	15	1.20	3.44	2.48	7.12
B, ¹ unaerated	5	6	8	19	32	12	15	17	16	16	1.37	3.70	2.48	7.55
G, aerated	4	5	13	34	73	12	15	22	28	33	3.70	12.17	12.12	27.99
B, aerated	4	6	15	42	85	12	17	25	32	35	4.32	12.18	14.25	30.75
Control, unaerated	5	7	11	23	32	13	15	16	16	16	.89	2.47	2.48	5.84
Control, aerated	4	5	17	45	90	11	11	32	35	35	5.29	12.43	16.73	34.45
Uncovered:														
G, unaerated	4	7	14	28	58	9	10	28	34	36	5.18	9.51	9.39	24.08
B, unaerated	5	8	20	45	61	11	21	29	34	35	4.17	8.16	8.90	21.23
G, aerated	4	4	4	6	17	8	9	8	10	28	2.57	8.26	4.19	15.02
B, aerated	4	6	6	7	22	8	9	8	13	31	3.59	8.87	5.71	18.17
Control, unaerated	5	7	15	38	62	12	15	27	33	36	4.24	7.76	11.60	23.60
Control, aerated	4	4	5	7	25	8	9	9	12	28	2.50	8.05	5.05	15.60

¹ G represents green algae; B represents blue-green algae.

In this series, an attempt was made to prevent the uncovered cultures inoculated with *Nostoc* from being contaminated by green algae from the air. Glass wool was packed about the stems of the plants and into the other holes in the corks, but it proved inadequate for the purpose. Green algae were soon observed in these cultures, and in a short time became as abundant as those in the jars that had been originally inoculated with green algae; thus any effects that might have been due to *Nostoc* were wiped out or rendered unobservable. There were no significant differences between the cultures with unicellular and those with filamentous green algae, and therefore they will not be dealt with separately.

Study of the data shows that the greatest difference again lay between the aerated and unaerated plants (fig. 4) in covered jars. The plants in the covered, aerated jars averaged 31.06 gm. and those in the covered, unaerated jars 6.84 gm., a ratio of 4.5 to 1. This agrees with the results of the previous experiment, although the difference is not as large as in the latter. This is no doubt due to the fact that in the first experiment the nutrient solutions were renewed three times, while in this experiment there was only one renewal.

A comparison of uncovered, unaerated cultures with the covered, unaerated cultures shows an average weight of 22.97 gm. for the uncovered as compared with 6.84 gm. for the covered jars, a ratio of 3.3 to 1. This confirms the effect observed before, namely, the beneficial effect of aeration by algae on the growth of the plant.

In comparing the uncovered, unaerated with the uncovered, aerated cultures, there appears to be a discrepancy between the results in this experiment and those recorded in the previous experiment. In this experiment the unaerated plants (fig. 5) made better growth than those aerated, the average weights being 22.97 and 16.26 gm. respectively, a ratio of 1.4 to 1. In the previous experiment, the ratio (with the dicalcium phosphate solution) was 1.3 to 1, but with the advantage in favor of the aerated cultures. This apparent discrepancy can be explained by examination of the rate of growth of the plants in uncov-

cred, aerated cultures, as revealed in the record of height and length of roots throughout the period of the experiment. These data show that the plants remained practically at a standstill until the early part of April, but the measurements of April 15 show a marked increase, which was accelerated within the remaining 2 weeks the plants were



FIGURE 4.—Tobacco plants placed in culture solution (C_2) February 6, 1938 (photographed May 4, 1938): A, Culture solution darkened and aerated; B, culture solution darkened, unaerated.

allowed to grow. Until these plants started normal growth, they exhibited the familiar symptoms of iron chlorosis, and this in spite of the fact that iron was added to these cultures at the rate of 2 cc. of solution per day for a period of 2 weeks. It is possible that the high pH value of the solution, due to the large amount of calcium carbonate present, caused precipitation of the iron or otherwise rendered it unavailable. Although all the cultures had the high amount of calcium carbonate, it seems reasonable to suppose that, at least in

those uncovered cultures that were not aerated, the lack of agitation of the solution aided in causing the iron to remain more available, since in unagitated solutions the upper layers do not exhibit the chemical concentration that occurs in the lower layers. The fact that growth



FIGURE 5.—Tobacco plants placed in culture solution (C_2) February 6, 1938 (photographed May 4, 1938): A, Light admitted to aerated solution; B, light admitted to unaerated solution.

proceeded normally in covered cultures that were aerated indicates that there may be a light factor involved also. The conditions affecting the intake of iron by plants in solution cultures are not well understood. Under a given set of conditions the plants may start normal growth and continue without interruption; under what are apparently the same conditions, they may develop symptoms of iron chlorosis from which they may not recover for several weeks, even though iron be added every day or two. In the experience of the writers, iron chlorosis cannot be corrected within 24 to 48 hours, as has been stated

by some investigators. However, it was observed during these and other experiments that intake of iron at the start of the experiment is made more certain if the culture is not at first aerated artificially.

The results of the experiment with the blue-green alga *Nostoc muscorum* in solutions containing no nitrogen are shown in table 8.

TABLE 8.—Average measurements during growth period and air-dry weights of tobacco plants grown in nutrient solution with no added nitrogen, with and without green algae and the blue-green alga *Nostoc muscorum*

Treatment	Average height of plants					Average lengths of roots					Average air-dry weight per plant			
	Mar. 4	Mar. 18	Mar. 31	Apr. 15	Apr. 29	Mar. 4	Mar. 18	Mar. 31	Apr. 15	Apr. 29	Roots	Leaves	Stalks	Total
Covered:	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>
G, ¹ unaerated	5	6	7	8	8	21	35	52	68	80	0.36	1.09	0.30	1.75
B, ¹ unaerated	5	6	6	7	7	24	42	48	60	77	.35	1.02	.26	1.63
G, aerated	4	5	5	6	6	17	36	54	71	77	.31	.89	.19	1.39
B, aerated	5	6	6	7	7	14	31	48	58	69	.29	.81	.20	1.30
Control, unaerated	4	5	5	6	6	30	43	52	57	64	.24	.65	.18	1.07
Control, aerated	4	4	5	5	6	23	46	59	62	71	.27	.68	.18	1.13
Uncovered:														
G, unaerated	5	5	6	7	7	33	49	63	79	91	.27	.83	.20	1.30
B, unaerated	5	5	6	6	6	30	54	65	70	76	.35	1.0	.29	1.55
G, aerated	5	7	7	8	8	47	53	67	81	99	.46	1.24	.32	2.02
B, aerated	4	5	6	6	6	41	51	58	58	63	.55	1.13	.26	1.94
Control, unaerated	4	5	6	6	6	34	46	53	64	72	.26	.75	.19	1.20
Control, aerated	4	5	5	6	7	34	55	63	69	83	.45	.91	.24	1.60

¹ G represents green algae; B represents blue-green algae.

At no time during the experiment did any of the plants in this series show evidences of normal growth. They exhibited the characteristic symptoms associated with lack of nitrogen. The plants remained small, the leaves small and yellow, with firing of the lowest leaves. Study of the measurements of height shows that in only three groups did the average increase in height exceed 2.5 cm. The roots, however, increased very greatly in length, in a number of cases reaching a length of over 100 cm. and in one case a length of 120 cm. The tendency of the roots to elongate in the absence of nitrogen has been previously reported by McMurtrey.¹¹ However, while the roots increased greatly in length, their total number was much less, so that their total weight was small. This is shown in the data for dry weights, in which the average weight of roots per plant ranges from about one-fourth to one-half of a gram. On a percentage basis, however, these roots average over 23 percent of the total dry weight, while those in the nitrogen series average about 12 percent. Part of this increase may have been due to the weight of *Nostoc* on the roots, but the values for the cultures without *Nostoc* show that its added weight could not have been very great. The *Nostoc* grew in greatest numbers on the bottoms of the jars, where it formed rounded, more or less hemispherical, colonies with a lacy, much-branched structure, resembling miniature sponges. In no case were the cultures observed to form thin films such as were noted in the inoculating culture. They did, however, show a definite blue-green color that had not been observable in the parent culture. No colonies were formed in covered jars, and no growth was visible to the naked eye, although Allison¹² found that *Nostoc* is able to grow in subdued light, and even, for a time, in the absence of light, provided sugar is added to the nutrient solution. No growth of green algae was detected in any of the cultures in this series, either covered or uncovered.

¹¹ See footnote 2.

¹² See footnote 10.

The high pH value of the nutrient solution due to the calcium carbonate used in this experiment is favorable to the growth of *Nostoc*, according to Allison. Nevertheless, although these algae made some growth, the amount of atmospheric nitrogen fixed by them was evidently too small or was not released so as to make any significant difference in the growth of the accompanying tobacco plants. In one series there is a small apparent difference in favor of the *Nostoc* cultures over the controls, but this is offset by the fact that one series of green algae cultures gave a higher yield than either *Nostoc* or the controls. The differences are therefore judged not to be significant. The average increase in dry weight, based on a comparison with 50 sample plants selected and dried at the beginning of the experiment, was 0.302 gm. for the roots, 0.893 gm. for the leaves, and 0.196 gm. for the stems, or a total of 1.39 gm. per plant.

SUMMARY AND CONCLUSIONS

Tobacco (*Nicotiana tabacum* L.) plants were grown in three different nutrient solutions, three sources of phosphorus being used. The plants were grown in aerated and un aerated solutions, with and without green algae and with roots exposed to or shielded from light, in an arrangement whereby all possible combinations of these factors were effected. The nitrogen-fixing ability of the blue-green alga, *Nostoc muscorum*, also was studied under the conditions of these experiments. The rate of growth was recorded in measurements of height of plant, length of roots, and final weights obtained after oven-drying the plants.

Green algae function as efficient aerators of nutrient solutions up to the point where their numbers offer serious competition for the available nutrients. Artificial aeration apparently acts as a check upon the growth of algae.

Artificial aeration gives the greatest increase in growth of tobacco plants in solution cultures.

Green algae associated with light appear to have a protective effect on tobacco roots against infection by fungi. The evidence indicates that this may be both physical and chemical.

There is no evidence from these experiments that the growth of green algae is correlated with the hydrogen-ion concentration of the nutrient solution.

The assimilation of iron by the plant at the beginning of a water-culture experiment may be retarded by artificial aeration.

The phosphorus in tricalcium phosphate is available to tobacco plants in solution cultures provided the roots come into direct contact with it.

The use of nutrient solutions containing green algae, exposed to light and not artificially aerated, is believed to be of value in starting plants for use in water-culture experiments. In no case where these conditions obtained, have the writers so far failed to secure uniform starting and growing plants. Once such a start is obtained, later transfer to covered, artificially aerated cultures can be made without any set-back to the plants.

Blue-green algae were not able to fix sufficient atmospheric nitrogen, in the nutrient solution used, to affect the growth of tobacco plants.

MORPHOLOGY OF THE VEGETATIVE ORGANS OF SUGARCANE¹

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INTRODUCTION

In selecting a basis for the description of cultivated sugarcane (*Saccharum*) varieties, characters of the inflorescence (4)³ are ruled out, partly because in many localities numerous varieties bloom rarely, if ever, and partly because of lack of knowledge of the diagnostic value of flower characters.

Since the earliest records of sugarcane in literature (25), gross morphological characters have been used in describing varieties. Even Barber (7, 8, 9, 10), in his monumental work on sugarcane, relied mostly on characters of stem, leaf, and root. With increasingly wider knowledge of the plant, various investigators have made use, at one time or another, of more minute and microscopic features, such as number and disposition of root primordia in the root ring, arrangement of stomata, and character of stem epidermis. The arrangement of hair on buds and leaves received special attention from Jeswiet (15) in his *Morphology of Sugarcane*, a course of lectures designed originally for use by the scientific staff of the Java Botanical Garden, engaged in sugarcane studies. This work was the first of an extensive series of papers (5, 6, 11, 16, 17, 18, 19, 20, 21, 22, 23, 24, 30) dealing with the taxonomy of sugarcane varieties and of seedlings that originated at the Java station. While it treated of the vegetative organs of the plant in their entirety, it put greatest emphasis on the description of hair groups, thus emphasizing the importance of hair groups in the general classification scheme.

There is something very attractive about Jeswiet's method as a key for the difficult classification of sugarcanes. Smith (31) used it in his seedling studies at Hawaii, Fawcett (14a) in Argentina, and Cowgill⁴ on some of the New Guinea varieties in the collection of the Division of Sugar Plant Investigations. However, except for these workers, Jeswiet's hair-group method has found few adherents, partly because the method is not practical as a ready means for the field identification of cane varieties and partly because, within a variety, seldom more than 50 percent of the hair groups are present on every bud and show a minimum amount of variation. It is, however, a

¹ Received for publication December 1, 1939.

² Credit is due Mrs. Eugenia Artschwager for preparation of the drawings.

³ Italic numbers in parentheses refer to Literature Cited, p. 547.

⁴ Cowgill, H. B. Unpublished data.

useful auxiliary method for the identification of varieties to be used by technically trained persons. Greater constancy, according to De Calvino (12) and Artschwager (2), is found in the character of the stem epidermis and, to a certain extent, in the structure of the leaf blade and leaf sheath (26).

The large collection of original importations by the Division of Sugar Plant Investigations gave the writer an opportunity to continue the extensive studies of sugarcane varieties interrupted by the untimely death of Cowgill, and to evaluate the usefulness of hair groups on material other than that used by the workers at the Java station. It is best to base identification upon the greatest possible number of characters, including those of external form, as has been done in the past. For this reason, characters such as shape, size, and color of internode; bud furrow; shape and size of buds and their hairiness; and form and size of ligule, auricles, and collar received as careful treatment as special methods, either already listed or newly tried, permitted.

As the work progressed, the need for a descriptive outline (3) more complete in its scope than the one recommended by the International Society of Sugar Cane Technologists (27), made itself felt. In the preparation of the new outline, the use of diagrammatic drawings illustrating type patterns of different characters was found helpful, making the data more uniform and more readily accessible for statistical studies. The hair-group numbers of Jeswiet have been retained, though with reservation; they often have great practical value, even though their regional designation may not always be acceptable.

MATERIALS AND METHODS

The material for study came from the United States Sugar Plant Field Station at Canal Point, Fla. All characters were studied and illustrations prepared from fresh material. For quick and easy reference, various parts of the plant, such as node, sheath base, ligule, dewlaps, auricles, and buds, were photographed on paper. The buds were always magnified six times and the photographs subsequently redrawn in order to show the hair groups on both surfaces to better advantage. The epidermis of stem, leaf, and sheath was easily stripped off after treatment with nitric acid. To obtain uniformly good results, it is best to dilute the concentrated acid slightly when treating leaves and to interrupt the heating before the acid comes to a boil. Removal of the stem epidermis necessitates the use of concentrated acid, to which a few crystals of potassium chlorate may be added. In a few varieties the stem epidermis does not strip easily, but even though some cortical tissue may remain attached it is usually possible to make the necessary observations and measurements and determine the epidermal pattern. To obtain better differentiation, it is advantageous to mount the stripped epidermis in zinc chloriodide. The inner stem structure may be studied from unstained hand sections, though previous treatment with phloroglucin in alcohol and hydrochloric acid greatly simplifies the task. Ligules and auricles may be detached and mounted in dioxane balsam without preliminary treatment; such slides are permanent and may be filed for future reference.

MORPHOLOGY OF THE STALK OR CJLM

Sugarcane is a tall, perennial, gregariously growing grass, with the culms bunched in stools or evenly scattered, sometimes provided with long creeping rhizomes.

THE INTERNODE

GENERAL MORPHOLOGY

The length and thickness of the stalk is determined, morphologically speaking, by the size and number of nodes and internodes composing it. The number of these stem units varies with and within a given variety. They are longest in the middle of the stalk and for some distance remain fairly even in size. Generally, the length and thickness of the internodes decrease from the middle part of the stalk in both acropetal and basipetal directions. The internodes are shortest at the very base of the stalk, and their shape is usually obconoidal. Sometimes extremely short internodes are interpolated between normal ones; at other times, especially when the growing season has been marked by extremes in weather, a few exceptionally long internodes may be found.

The internodes assume their characteristic varietal shape before they reach maximum development. Their cross-sectional area is round, in some varieties oval, and their disposition on the stalk is alined or zigzag (staggered).

The most common forms of internodes are illustrated in figure 1.

In the cylindrical internode (fig. 1, *A*) the cross section remains the same, while in the tumescent type (*B*) it is greatest near the middle, decreasing gradually in either direction. The bobbin-shaped type (*C*)

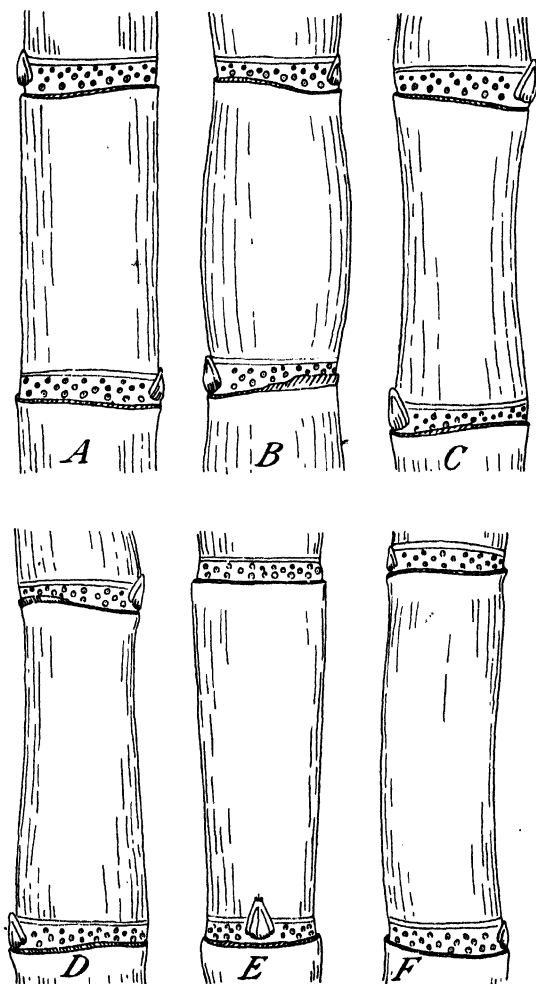


FIGURE 1.—Internode patterns: *A*, Cylindrical; *B*, tumescent; *C*, bobbin-shaped; *D*, conoidal; *E*, obconoidal; *F*, curved.

is the exact reverse of the tumescent internode, being thinnest in the middle. In the conoidal form (*D*) the diameter decreases acropetally, while in the obconoidal type (*E*) the greatest diameter is immediately below the wax band and the smallest immediately above the intercalary zone, or growth ring. The conoidal internode frequently has a bulge or shoulder directly above the growth ring on the side opposite the eye, and certain cylindrical internodes become more or less depressed on the bud side and convex on the opposite side (*F*).

Internode patterns have a certain value in classification, provided the material is from plants developed under favorable conditions. An abnormal environment is often responsible for atypical forms, which may occur on the same stalk with normal internodes.

Color of the stalk is regarded by many experienced workers as an important character, even though Jeswiet (15) concluded that it would never become a basis for classification of cane varieties.

In noting the color, reference should be made to mature and exposed internodes that have not yet become faded or discolored. Color is a very obvious character; although it may vary from internode to internode, the general hue is typical for a given variety. To the inexperienced, it may seem to be a very elusive character, since environmental factors often greatly influence its expression. According to Earle (14) and other authors, many sugarcanes that are normally green show a pronounced reddish or brownish flush on exposure to light and air.

Striking color differences are often noted in freshly cut stem sections. In some varieties, the color of the cross section is uniform to the very center; in others, the outer zone is of a different hue, often a deep green, while the center is grayish, ivory, or whitish if the variety is pithy.

Of interest, and often a character of importance, is the bud furrow, or eye groove (fig. 2), a depression in the stalk beginning in the root band underneath the bud and extending upward. The bud

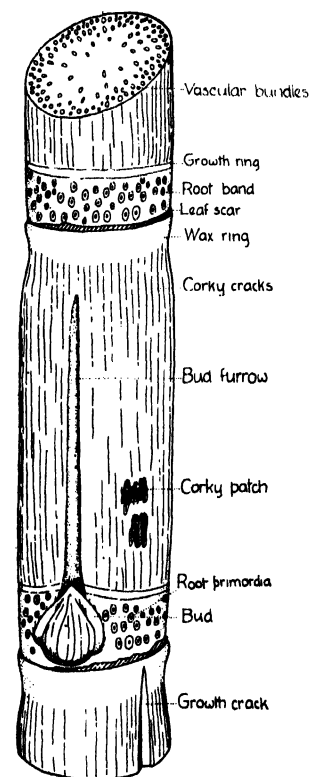
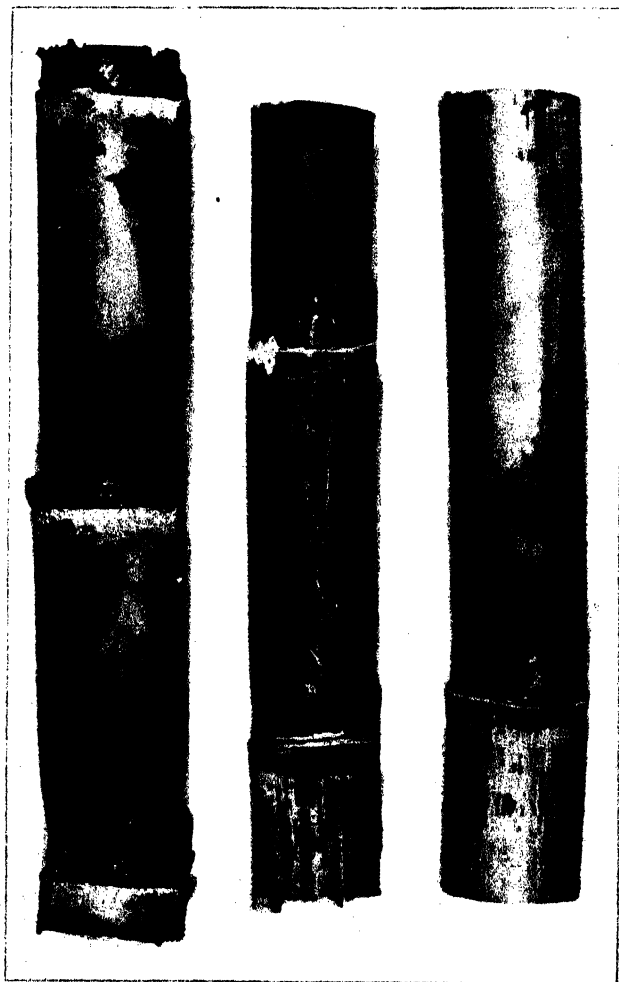


FIGURE 2.—Diagrammatic drawing of node and internode.

furrow may be either wide or narrow, deep or shallow, long or short. It may be absent or show as a slight flattening of the stem above the bud or be limited to a shallow cavity. Sometimes the furrow is narrow and deep and extends along the entire internode, with the bud embedded in a rather deep depression. The depth and width of the furrow may be uniform throughout, though more often it tapers in the direction of the wax ring, gradually becoming narrower and shallower. The color of the bud furrow is usually like that of the rest of the internode but occasionally different.



Mature internodes of variety 28 N. G. 204, showing corky cracks and progressive stem discoloration, which give the surface the appearance of dead stalks.

The surface of the stalk is almost always glabrous. A distinct pubescence was found in 75 Lehu (Imp.⁵ 736) briefly described by Moir (28) in 1932, and in New Caledonia 32 (Imp. 892). Occasionally the smoothness of the surface is broken by the appearance of corky cracks or growth cracks.

Corky cracks, or ivory markings, are small longitudinal crevices in the epidermis (fig. 2). In old stalks they may appear rough and become confluent, forming corky patches. The cracks are at first the color of the internode and rather inconspicuous; later they turn whitish and may be edged with a different color. Often they are found only in older internodes, in the zone of the wax ring and immediately below, and also along the bud furrow. They may be very irregular and scattered, absent from some internodes while present in others. Occasionally their occurrence is limited to those parts of the stalk that have been much exposed to the sun; here they often unite to form gray spots or patches, which sometimes unite in a grayish ring below the wax band.

Often the surface of entire internodes becomes progressively discolored or brown (pl. 1) until the cane looks like a dead stalk. This appearance is effected by color changes in the hypodermal cells and usually takes place independently of the formation of cracks in the epidermis.

Deeper longitudinal cracks, which extend into the tissue of the internode, are the so-called growth cracks (fig. 2). These often reach into the intercalary zone but never into the adjacent internode. They may occur along with corky cracks and are generally considered a fairly reliable character.

All parts of the stalk, with the exception of the growth ring, have a coating of wax which, in the region immediately below the sheath base, forms a more or less conspicuous white band. The waxy coating may be even and thick, often dense enough to hide the color in the young internodes, or it may be thin and uniform, giving the surface a glazed appearance. In some varieties, bloom is practically absent, and then even the wax ring is inconspicuous. In some reddish or purplish canes, the upper half of the internode is densely covered with wax that gradually becomes thinner and more transparent and disappears altogether in the basal region. When the waxy coating is very heavy, it may peel off in places, imparting to the surface of old stems a peculiar striation. In internodes that are no longer protected by the sheath and sometimes also in younger internodes, the wax may become discolored. This change in appearance may affect the entire internode, or else the discolored areas may form irregular patches varying in color from dirty white to almost black. The discoloration is usually caused by the superficial growth of certain molds.

The wax deposit is always thickest and whitest below the sheath base. Usually there is a distinct division between wax ring and general bloom, but sometimes this demarcation is faint or disappears in canes where the bloom is heavy. Then the result is a gradual and complete merging of the wax of the ring with that of the general bloom. In most cases the wax ring is conspicuous and very white. Its width is subject to much variation and may be considered a varietal character. The wax ring is always narrow in young internodes but is

⁵ Imp. = Importation number assigned by the Division of Sugar Plant Investigations.

usually set off more distinctly than in older internodes. In a few varieties the ring is distinctly striated (New Caledonia 92, Imp. 913). Sometimes the wax of the ring, like that of the general bloom, becomes discolored in older internodes and may peel off in patches or entirely.

The tissue of the internode consists of vascular bundles embedded in parenchyma. The bundles are rather widely spaced in the central part. Occasionally the center becomes pithy, and in certain wild forms a distinct cavity develops. The number of bundles increases centrifugally. At the periphery they lie very close together often forming a solid ring (pl. 2, *A, B*). All bundles are surrounded by a sclerenchymatous sheath, which is most strongly developed in the peripheral bundles. The outermost ring may be composed altogether of small bundles or of small bundles alternating with larger ones (*B*). Relative size and spacing of the bundles is not correlated with thickness of the internode.

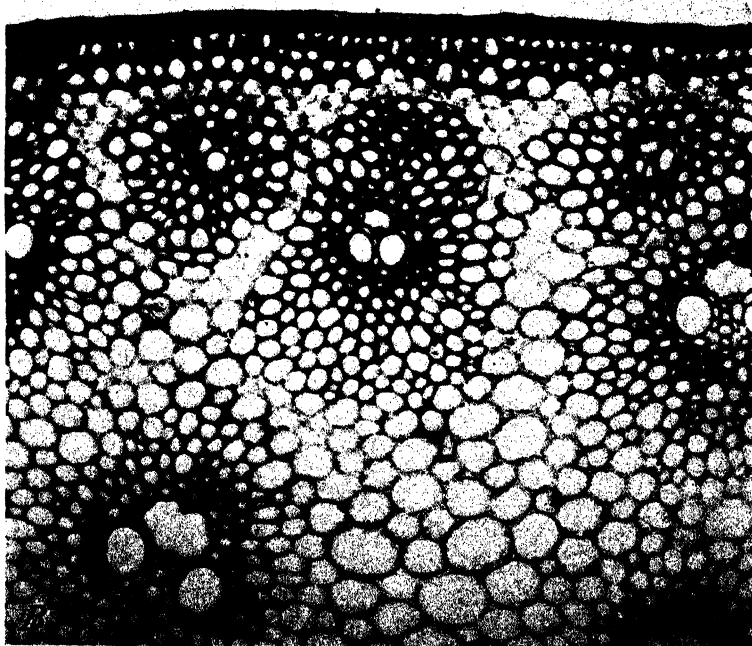
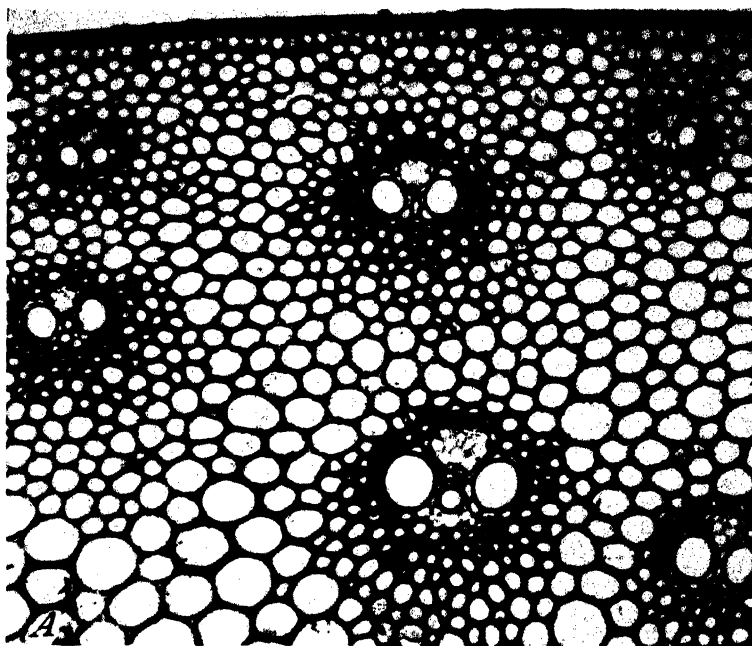
Table 1 indicates the extent of relationship between bundle density in the peripheral ring and thickness of internode. One would expect a larger number of bundles per unit area in the thin canes than in the thick-stemmed forms, yet in many of the very thin wild canes the bundles are rather widely spaced. The number of bundles in the outermost ring is often, though not always, influenced by their size. If the number is large, all peripheral bundles are likely to be small, and conversely a very small bundle number indicates that at least some of the peripheral bundles are of the large type. Although there is some variation within a variety, bundle density within the peripheral ring and relative size of these bundles constitute a valuable character.

TABLE 1.—*Relation between thickness of stem, total number of bundles, and number of bundles in peripheral ring as seen at the widest diameter of a microscopic field covered by a 16-mm. objective and a $\times 6$ eyepiece.*

Variety	Importation No. ¹	Diameter of stem	Bundles		Variety	Importation No. ¹	Diameter of stem	Bundles	
			Total	Peripheral				Total	Peripheral
			Number	Number				Number	Number
		Millimeters					Millimeters		
Shantir Barhni.....	142	7	10	5	New Caledonia 31...	891	36	11	4
U. S. Imp. 229.....	229	11	7	6	New Caledonia 32...	892	39	8	3
Tabongo.....	578	11	10	4	New Caledonia 33...	893	36	10	5
Hemja.....	234	22	10	6	New Caledonia 40...	897	38	10	5
Nagori.....	235	17	9	5	New Caledonia 42...	898	34	6	4
Manjav.....	95	24	14	6	New Caledonia 50...	902	33	9	4
Kinar.....	6	21	7	3	New Caledonia 51...	894	43	9	4
Merthi.....	7	21	11	3	New Caledonia 53...	895	31	12	5
Fiji 3.....	862	15	14	5	New Caledonia 59...	899	33	10	5
Hawaiian Original 6	825	35	14	6	New Caledonia 64...	901	24	12	6
Hawaiian Original 20	828	29	13	6	New Caledonia 74...	903	35	9	4
Hawaiian Original 38	832	34	12	6	New Caledonia 76...	904	33	9	5
Hawaiian Original 43	836	34	14	5	New Caledonia 78...	905	38	10	4
New Caledonia 20...	885	28	13	9	New Caledonia 80...	906	29	9	4
New Caledonia 21...	886	34	7	3	New Caledonia 81...	908	40	10	5
New Caledonia 25...	888	38	10	5	New Caledonia 83...	910	29	12	5
New Caledonia 30...	890	25	11	8	New Caledonia 92...	913	29	12	5
					New Caledonia 93...	914	29	7	4
					New Caledonia 99...	917	38	11	6

¹ Assigned by the Division of Sugar Plant Investigations.

The peripheral ring is separated from the epidermis by a very narrow cortex, not more than four cells wide. The cells of the two rows next to the epidermis are small, usually thick-walled, and in



A, Partial stem section of U. S. 859. Peripheral bundles well separated; peripheral cells and surrounding parenchyma thick-walled and heavily lignified. B, Partial stem section of P. O. J. 2714. The sheaths of the outer row of bundles are very large and confluent. Both $\times 300$.

mature stalks heavily lignified. Adjacent to this layer are one or two rows of thin-walled parenchyma cells with chloroplasts. The walls of these cells remain cellulose even in old and mature stalks.

The central stem bundles have a bundle sheath that is relatively wider at the phloem and xylem poles than at the sides, where it is usually restricted to one or two layers. The width of the sheath at the two sides is more or less constant, but there may be a considerable difference in the thickness of the cell walls and in the degree of lignification. The parenchyma cells which constitute the filler between the bundles, are usually thin-walled; but in some varieties, and especially in wild canes, they may become fairly thick-walled and even lignified. Commonly there are no color differences within the tissue of the vascular bundles, but Jeswiet (15) reports for some varieties sheaths of a characteristic dark-brown color.

STRUCTURE OF STEM EPIDERMIS

TYPES OF CELLS

The essential structure of the stem epidermis has been described in an earlier publication (2). It is in agreement with the type of structure that obtains in grasses in general. The epidermis consists of a single layer of different kinds of cells, of which each kind may vary as to size and number but, on the whole, forms a uniform pattern characteristic of the variety. There are three kinds of cells: Long cells, cork cells, and silica cells. Frequently a few scattered stomata are found, and there is a definite pubescence present in New Caledonia 32 and in 75 Lehu.

The long cells form four-sided prisms with undulating, strongly silicified walls. In some varieties many of these cells are completely filled with crystal sand (a form of calcium oxalate crystals). There is considerable variation in the length of these cells and in the slope of their end walls; the latter are commonly straight, though often pointed. The variation in width of the long cells appears very slight in many cultivated varieties because of the presence of intermediate forms, but it is quite apparent when varieties with very wide and very narrow cells are compared. There is no correlation between cell width and stem thickness; in many thin-stemmed wild canes the epidermal cells are very wide, while in some thick-stemmed noble varieties these cells are relatively narrow.

Exceedingly narrow epidermal cells were found in Fiji 2 and Fiji 3, but here again the thinner-stemmed variety had wider cells than the thicker-stemmed one. Tabongo (*Saccharum spontaneum*), with a stem only 11 mm. across, had as wide a cell as New Caledonia 51, which has a stem four times as thick (table 2).

The cork cells are relatively thin-walled and suberized. They occur in a wide variety of forms: Squarish, rhomboid, reniform, elongate-rectangular, and long- or short-pointed. Often a single pattern prevails, but sometimes different patterns are seen in a single microscopic field. Cork cells often appear singly, separated from one another by long cells; frequently they are grouped in twos and threes. Most often, however, they are adjacent to a silica cell, forming a typical short group so characteristic of the grass epidermis.

TABLE 2.—*Relation between thickness of stem and width of epidermal cells*

Variety	Importation No. ¹	Diameter of stem	Width of epidermal cells	Variety	Importation No. ¹	Diameter of stem	Width of epidermal cells
		Millimeters	Microns			Millimeters	Microns
Shantir Barhni.....	142	7	10.7	Hawaiian Original 24.....	827	38	11.0
U. S., Imp. 229.....	229	11	11.8	Hawaiian Original 38.....	832	34	8.4
Tabongo.....	578	11	13.7	New Caledonia 20.....	885	28	10.3
Hemja.....	234	22	11.0	New Caledonia 25.....	888	38	9.0
Nagori.....	235	17	11.0	New Caledonia 30.....	890	25	10.7
Manjav.....	95	24	11.4	New Caledonia 31.....	891	37	13.2
Kinar.....	6	21	12.2	New Caledonia 32.....	892	39	12.2
Merthi.....	7	21	12.7	New Caledonia 33.....	893	36	11.0
Fiji 2.....	861	22	7.0	New Caledonia 40.....	897	38	9.4
Fiji 3.....	862	16	9.2	New Caledonia 51.....	894	43	14.3
Hawaiian Original 6.....	825	35	9.7				

¹ Assigned by the Division of Sugar Plant Investigations.

The silica cells are much more uniform than the cork cells. They are usually biscuit-shaped, with a constricted center and overhanging margins, and with the long diameter parallel to the long axis of the stem. Silica cells rarely occur alone between long cells but are rather associated with cork cells. In some varieties they are almost altogether absent, and in many they are lacking from about 50 percent of the short groups.

Stomata are comparatively few or entirely wanting. There is a certain degree of regional distribution. Frequently stomata appear in broken rows wherever they are relatively numerous; when scarce, their location is quite erratic. In one variety with a vertically striped wax ring, stomata were found almost exclusively in the areas where there was no wax.

The following criteria proved to be of value in classifying varieties on the basis of epidermal patterns of the stems:

(A) Primary characters.

- (1) Absence of silica cells.
- (2) Prevalence of pointed cork cells.
- (3) Occurrence of cork and silica cells in multiple pairs.
- (4) Prevalence of very long rectangular cork cells.

(B) Secondary characters.

- (1) Relative abundance of stomata.
- (2) Width of long cells.
- (3) Average number of short-cell groups in a microscopic field.

EPIDERMAL PATTERNS

Type 1.—Cork and silica cells in single pairs alternating with long cells. The latter have usually straight-running, undulate vertical walls and straight or somewhat sloping end walls (pl. 3, A).

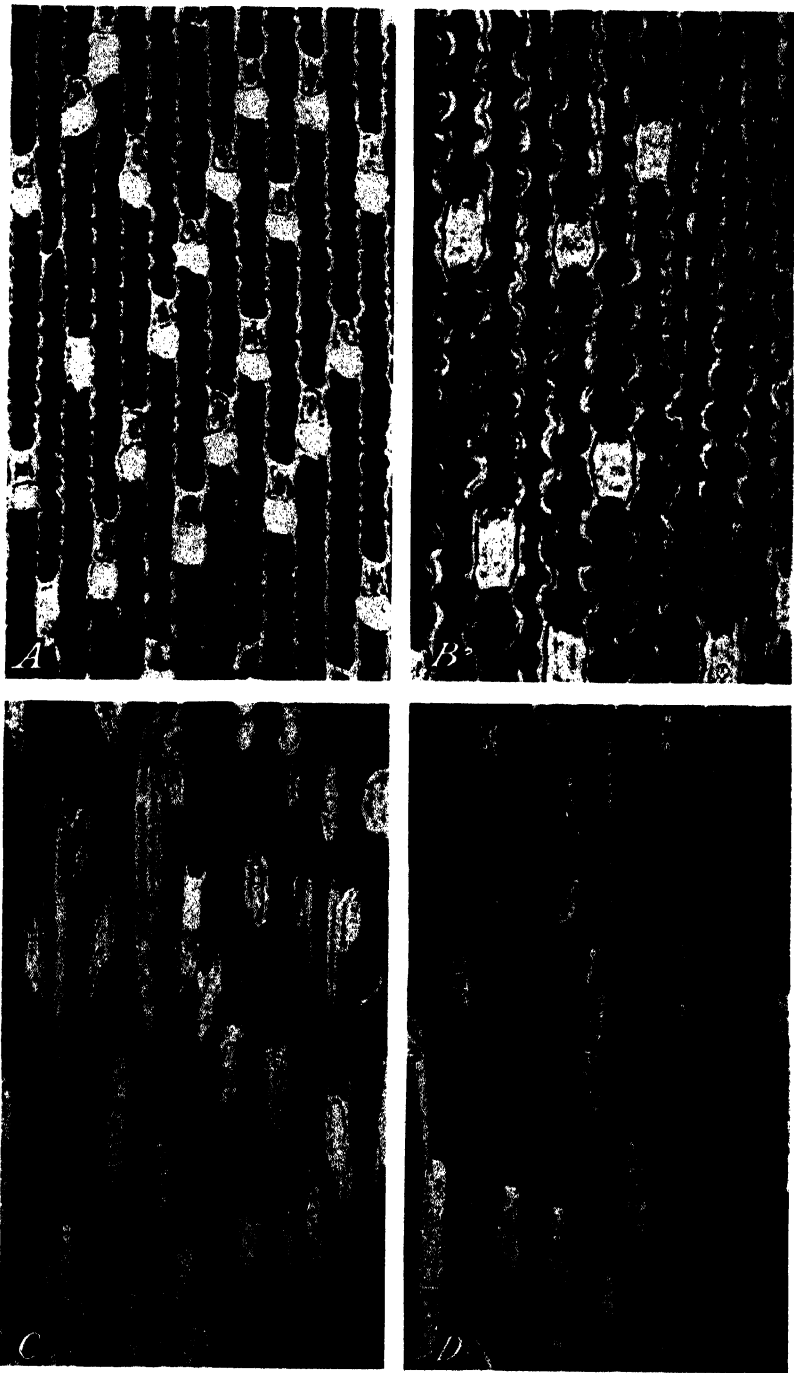
Type 2.—Silica cells wanting or very scarce. Cork cells solitary or in groups; squarish, rhomboid, or, in some varieties, greatly pointed and interspersed with the narrow elongate-rectangular type (pl. 3, B).

Type 3.—About 50 percent of the short-cell groups lack silica cells; the shape and distribution of the different cell forms show much variation otherwise.

Type 4.—Practically all cork cells are pointed. The vertical walls of the long cells are often curved and their end walls pointed. There is much difference in the width of the long cells. The number of short-cell groups lacking silica cells may show considerable variation. If this number is approximately 50 percent, the pattern formula would be expressed as 4+3 (pl. 3, C).

Type 5.—All cells are rather narrow and there is a predominance of the elongate-rectangular type of cork cell, occurring singly or in short connected rows.

Type 6.—The short-cell groups are very numerous and always occur in multiple pairs (pl. 3, D). The cork cells are usually somewhat elongate, squarish, or rhomboid.



Surface view of stem epidermis illustrating (A) Pattern 1, (B) pattern 2, (C) pattern 4, (D) pattern 6. All $\times 500$.

The different epidermal patterns are not equally abundant. Table 3 shows their frequency distribution in a random collection of 100 varieties.

Types 1, 2, and 4 are the most frequent. If only single characters and not combination types are used, the numbers listed under 2+4 and 3+4 would have to be redistributed. They could all be put in type 4, raising the frequency distribution in this group to 35; or, if we consider type 2 more fundamental than type 4, the nine varieties listed under 2+4 could be added to type 2, raising the frequency distribution of that group to 26. However, combination groupings are more descriptive and characterize the variety better. They are best used when characters are not strongly developed; this applies especially to pattern 4. Long- and short-pointed cork cells, which characterize this pattern, may be relatively scarce or abundant. If their number is small, it is best to designate the epidermis where they occur by a combination formula.

TABLE 3.—Frequency distribution of varieties according to epidermal pattern

Item	Pure type						Combination type	
Pattern type.....	1	2	3	4	5	6	2+4	3+4
Frequency.....	20	17	14	16	5	9	9	10

The relative value of the epidermal pattern in classification may be debatable or even questionable if applied to a rather homogeneous natural group. It failed to give satisfactory results for Panje (29) in his studies of *Saccharum spontaneum*. But even here, Burma (*S. spontaneum*) stood out among all other forms by the fact that practically no silica cells occurred in its epidermis. The epidermal pattern may be used only as an auxiliary method in a general classification scheme, but sometimes it alone suffices to make a doubtful grouping positive, especially if the forms in question have entirely contrasting epidermal patterns.

THE NODE

The node is usually somewhat thinner than the internode and exhibits the following regions: Node proper, a zone of anastomosing and horizontally running leaf-trace bundles (1), limited above by the sheath scar; and root band, which contains the bud and several rows of root primordia. The growth ring, or intercalary zone, connects node with internode.

GROWTH RING

The growth ring is located directly above the root band and stands off from the latter and the internode above as a rather narrow band. It differs somewhat in color from the rest of the stem and lacks the wax deposit found on other organs of the plant.

The growth ring commonly runs horizontally or turns slightly upward above the bud or occasionally is very much arched in this region. Usually it is flush with the adjacent internodal tissue but may be somewhat depressed, most noticeably so in young stems. In older stems, it frequently bulges as a narrow ridge on the bud side or around the entire circumference (pl. 4, D).

In some varieties the growth ring is narrow and inconspicuous; in others, quite broad (pl. 4, *B*), a characteristic of many wild canes. Sometimes it is unequal in width all around or wider on one side than on the other; this occurs where the stalk has bent or straightened itself through the activity of the cells of the intercalary meristem.

The color of the growth ring varies in immature and grown stalks. It is at first somewhat different from the internode; on maturing, it may retain this difference or become concolorous with the internode.

ROOT BAND

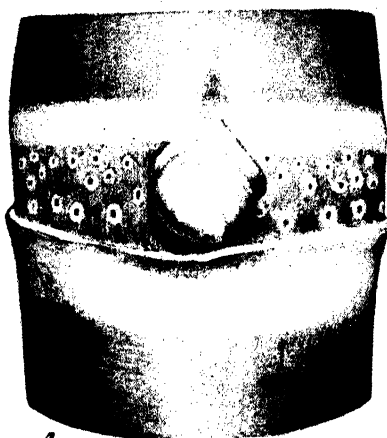
The root band is limited by the sheath scar below and growth ring above and contains, besides the bud, several rows of root primordia. It is almost colorless in young nodes but eventually becomes more or less concolorous with the rest of the stem. It varies in height, being usually taller on the side of the bud than on the opposite side. Sometimes it is slightly or prominently curved on the bud side. The root ring has a distinct coating of wax, which at times may be very heavy; this waxy coating often bears an imprint of the veins of the addressed leaf sheath. Hairs on the root band were observed in New Caledonia 32 and 75 Lehu, two selections that are prominently hairy all over the stem.

The shape of the root ring is often characteristic of the variety. The most common form is cylindrical. Sometimes the ring is cylindrical on the bud side and obconoidal on the opposite side. Often obconoidal internodes have a conoidal root band, this shape being equally pronounced in tall and narrow root bands. In stems with prominent and swollen growth rings, the root band is frequently constricted (pl. 4, *D*) or depressed only on the side opposite the bud. Tumescence root rings are not uncommon; the swelling may be regional, often restricted to the bud zone. Obconoidal root bands are often associated with conoidal and sometimes with bobbin-shaped internodes.

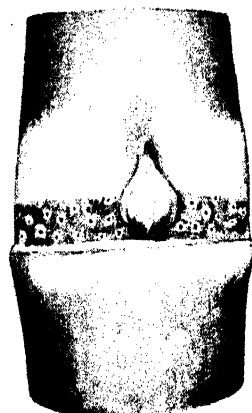
The root primordia are the initials of secondary roots. They occur in one or in several regularly or irregularly arranged rows. In wild canes, only one or two rows are found, even when the root band is rather tall. The number of rows of root primordia in a root band is usually greater on the side with the bud, although this region is not always broader than the side opposite the bud. In some narrow root bands with numerous rows of root primordia, the upper row may continue above the eye. Sometimes the root primordia in the different rows are disposed vertically, one above the other, but more often they are arranged in an oblique ascending series. Frequently the arrangement is irregular (pl. 4, *C*), a condition more common in root bands where the root primordia are numerous than in bands where they are not crowded.

The root primordia in a row are generally uniform in size; the top row in the root band contains the smallest primordia and the bottom row the largest (pl. 4, *A*). In a given stalk, the basal nodes have fewer rows of root primordia than the nodes nearer the middle. There is no further decrease in the number of rows toward the top.

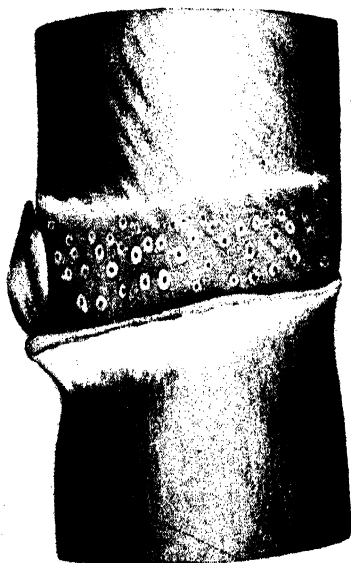
Each root primordium has a dark center and a light-colored or "halo." The dark center constitutes the root cap, which is intensely colored. In young nodes the primordia are comparable as dark points in translucent surroundings. The colored



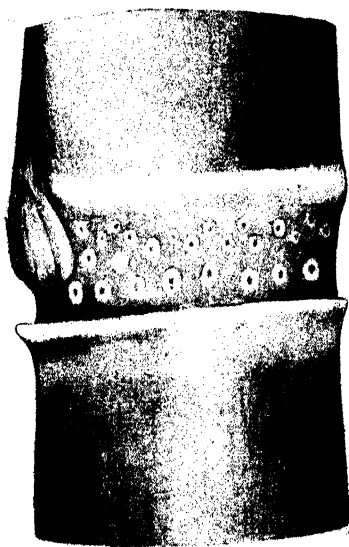
A



B

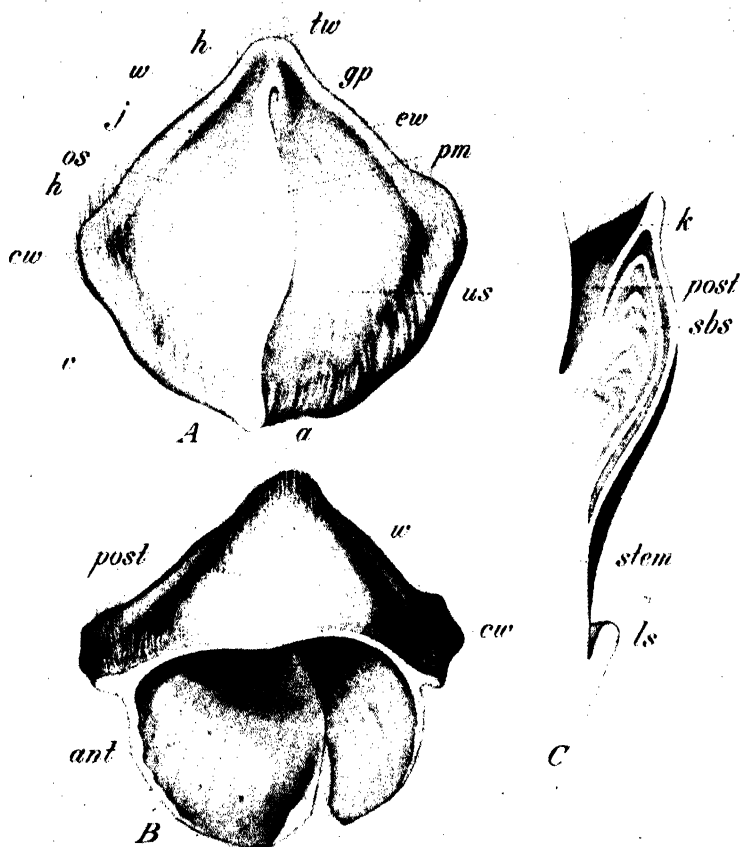


C



D

of nodal region: A, Growth ring medium wide; root band with large, spaced root primordia. New Caledonia 24. B, Growth ring very narrow; root band very narrow with small, more or less crowded root primordia. Haak Kwat Che. 49. C, Growth ring very narrow; root band broad with many irregular, crowded root primordia; sheath scar protruding and spreading under growth ring. Haak Kwat Che. D, Growth ring medium wide and swollen; root band with large, widely spaced root primordia. Bud is inserted above sheath scar and projects above growth ring. Hawaiian Original 39. All $\times 1\frac{1}{2}$.



A, Anterior or front side of prophyllum; *B*, posterior or back side of prophyllum; *C*, view of bud cut longitudinally to show structure and attachment of prophyllum. In its entirety, the prophyllum forms a hood, with the front side the larger and composed of two overlapping halves, and with the posterior side entire. All $\times 7$. *a*, Appendage of overlying half of anterior side; *ant*, anterior side of prophyllum; *cw*, corner of wing; *ew*, edge of wing; *gp*, germ pore; *h*, hood; *j*, juncture of wing with anterior side; *k*, keel; *ls*, leaf sheath scar; *os*, overlying half of anterior side; *pm*, parenchymatous margin of overlying half of anterior side; *post*, posterior side of prophyllum; *sbs*, second bud scale; *tw*, tip of wing; *us*, underlying half of anterior side; *v*, veins; *w*, wing.

is slightly raised and sometimes protrudes wartlike, especially in older nodes. The surrounding border is always lighter-colored than the center; its shape is round, occasionally oval. Its width varies from narrow to broad. The border, or halo, is usually flush with the surrounding tissue but is occasionally depressed, especially in young material. The root primordia remain dormant in many varieties, but in some they show a tendency to sprout and produce air roots while the stalks are still actively growing in the field.

THE BUD

GENERAL MORPHOLOGY

The buds, or eyes, of sugarcane are small conchate organs inserted in the tissue of the node just above the leaf scar. The visible part of the bud is the outermost bud scale, or prophyllum, composed of the sides and the wing. The prophyllum in its entirety forms a kind of asymmetrical hood (pl. 5, *A, B, C*) with the anterior or front side composed of two overlapping halves and with the relatively short posterior side entire and basally limited by a curved line at which it attaches to the tissue of the node.

The edge of the overlapping half of the anterior side is drawn out to form a membranous margin, which extends from the germ pore downward and frequently terminates in an appendage. As the bud matures, the tissue of the membranous border becomes dry and the basal appendage darkens and shrivels. In some varieties the membranous border is uniformly narrow throughout; and, if the degree of overlap between the two halves of the anterior side is small, the two margins occasionally alternate; i.e., the underlying margin becomes the overlying one for a short distance. Usually the membranous border is narrow only in the region of the germ pore, becoming gradually or abruptly broader at the base. The basal appendage projects in most varieties somewhat below the level of the bud insertion, but in some it is found rather high (fig. 3, *A*). It varies greatly in general appearance and prominence, even on the same stalk. Occasionally smaller accessory appendages are present on either the upper or the underlying margin of the scale. Unusual forms of such accessory lobes are described and illustrated by Jeswiet (23) for Black Cheribon and certain other varieties. In addition to the basal appendage, we find in certain varieties lateral outgrowths at any point of the membranous border (fig. 3, *B*). Of these, the oblique terminal outgrowth (fig. 5, *A, B*), which often covers the germ pore, is especially conspicuous.

The wing of the prophyllum is formed by the two flattened and beaklike keels (pl. 5, *C*). It varies in size and prominence, and its shape influences the shape of the bud. The boundary line between wing and sides, the so-called juncture, is not always clearly marked; but usually a more or less distinct groove, which is especially prominent in the basal corners, is present. In most buds the wing originates below the middle of the anterior side of the prophyllum (fig. 3, *A*); this is generally true of long-triangular and ovate buds. In other types the point of insertion may be intermediate or relatively high (fig. 3, *B*). The latter type of insertion gives rise to the more unusual

Relative size of wings varies greatly in different varieties. The wing may be uniformly wide over its entire length, or it may be wider or narrower at the base than at the apex; sometimes it is very irregularly lobed. Often the wing is very broad at the base and, in addition, is more or less prominently auriculate. However, well-developed au-

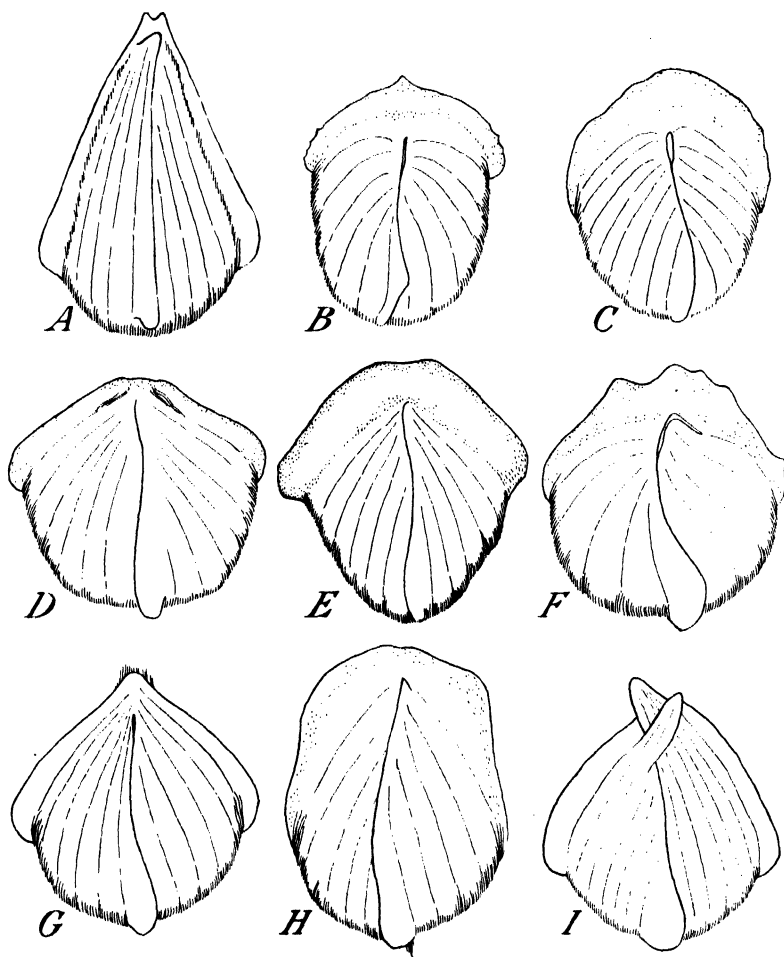


FIGURE 3.—Bud patterns: *A*, Triangular-pointed; *B*, oval; *C*, obovate; *D*, pentagonal; *E*, rhomboid; *F*, round; *G*, ovate; *H*, rectangular; *I*, beaked.

ricles are not restricted to basally wide wings. At times prominent auricles are found on only one side of the wing, being inconspicuous or absent on the other side. This is a phenomenon quite in keeping with the general tendency toward asymmetry in the development of the different organs of sugarcane.

The surface of the wing is usually flat; but in young buds, sometimes also in older ones, it is goffered or crimped. The edges of the wing also exhibit typical form patterns. Frequently the entire wing

is coarsely notched or emarginate; sometimes only the base is notched while the rest of the wing is entire. Quite often, however, the entire edge of the wing is smooth.

The apex of the bud has a characteristic shape, which is a part of the general bud pattern. The apex may be broadly triangular or crescentiform, occasionally with a flat or depressed truncate tip. Such forms are usually found in broad buds and in those where the wing is inserted high above the middle of the prophyllum. Very common are forms with a round- or sharp-pointed apex. If the tip of the prophyllum in either of these forms is prominently developed, the result is the beaked or rostrate type (fig. 3, *D*).

Usually the margin of the apex is like that of the wing, but sometimes it is quite different. For example, a prophyllum with otherwise smooth wings may have a tip that is deeply furcate, finely notched, or coarsely emarginate. In the broad crescent-shaped apex of certain buds there may be a single sharp projection, giving to the bud a mucronate appearance.

The anterior side of the prophyllum shows typical venation, which may be prominent or obscure. Sometimes the veins appear more conspicuous because of their contrasting coloration, as found in certain varieties. Usually the veins run almost parallel, converging in a gradual curve from base to apex. In round, and sometimes also in broad squarish buds, the veins approach the apex radially. There are, of course, many intermediate types.

The region where the two overlapping halves of the anterior side of the prophyllum unite is the location of the germ pore, a place where the growing shoot pushes through the protecting bud scales. Sometimes this place is marked by an irregular cleft (fig. 3, *F*); and sometimes it is covered over by the apical appendage of the membranous margin (fig. 3, *A*). In long buds, the location of the germ pore is near the tip; whereas, in round buds, in which the arrangement of the veins approaches the radial, the germ pore is central.

Much variation in both size and general appearance of buds is found not only in those of different varieties but frequently in those coming from the same stalk. However, if normally developed stalks are selected, varietal differences overshadow individual variation. Only fully developed buds should be chosen for comparison, preferably those coming from underneath fully developed leaves that are still attached to the plant. Depending on the variety, buds may be large or small, long or short, flat or plump. Young buds are always rather flat; later they become arched and attain a certain degree of fullness that is more pronounced in one variety than in another.

The tip of the bud may extend some distance above the growth ring (pl. 4, *D*), especially in large and elongated buds. In small, round buds and often in other types where the wing is inserted very high, the tip may reach to the intercalary zone (pl. 4, *A*) or stop short of it.

Sugarcane buds exhibit a most diverse variety of morphological forms; these have been loosely grouped under eight types. In selecting these patterns, little attention was paid to the appearance of the wings, although certain wing types are fundamentally associated with special bud forms. These bud patterns are illustrated in figure 3. The most common form is the ovate bud, which may be either elongate

(fig. 3, *A*) or broad. Quite common is the pentagonal form (*D*), usually associated with a high-inserted, rather broad wing. The oval (*B*) and obovate (*C*) types are less frequent. The ovate type has usually a high-implanted wing; in the obovate type, the position of wing insertion is less definite. Typical round buds (*F*) are not common; a related form, the rhomboid (*E*), is only occasionally found. At times the shape of the wing completely changes an otherwise typical bud pattern, as in figure 3, *H*, in which an abnormally developed wing changes an otherwise typically ovate bud into an aberrant rectangular form. Crossed beaked tips (*I*)⁶ produce a very striking effect and easily characterize a variety, provided this character is constant and not limited to a few buds on a stalk.

The color of mature buds that are still protected by leaf sheaths approximates that of the stalk. Contrasting colors are apt to occur in young buds; aging buds, regardless of previous coloration, are usually gray-brown. Red buds, and often green buds, are yellow when young, and an indistinct brown when aged. Often young buds are pale green with a contrasting reddish tint in tip and wing.

The buds are placed alternately on the stem, each node bearing a single bud. Buds are occasionally absent or underdeveloped in the basal part of the stalk, and occasionally a node is found bearing two buds in juxtaposition. Jeswiet (15) reports the occurrence of doubling due to splitting of the normal bud, and also the development of adventitious buds, which are either axillary or found on any part of the internode.

The buds are commonly inserted directly above the leaf scar, but in many wild canes and in some of their hybrids they occur some distance above, as high as the middle of the root ring. Occasionally, the buds appear to be inserted some distance below the leaf scar. Such canes usually have sagging leaf bases and protruding sheath scars.

Much variation is found in regard to the position of the bud on the stalk. Sometimes buds that are inserted rather low are located in a cavity and then seem very much appressed; this condition is often found in canes with a deep and well-developed bud furrow. Young buds are usually appressed, and in certain varieties even mature buds retain this tendency. When buds mature they frequently protrude, pointing away from the stalk at an oblique angle. Buds with a varietal tendency to protrude are associated with cane varieties in which the internodes are conoidal.

PUBESCENCE OF PROPHYLLUM

The buds of sugarcane, like those of other grasses, are covered with hair. In some varieties the buds are completely covered; in others the hairs are chiefly on the wings. Many cultivated forms are only moderately hairy, and some are almost entirely glabrous.

The hairs on the prophyllum are either long, white, often curved, and strongly appressed, or very short and of a brownish or blackish color. The brown hairs, which Jeswiet (15) regards as the primitive form, are often covered up by long white hairs.

The hairs are commonly concentrated in groups that are similar or identical in corresponding regions of the two overlapping halves of the

⁶ Redrawn from Jeswiet (25).

prophyllum. Some of the groups are again duplicated on the posterior side; i. e., certain groups are actually present in multiples of four, although Jeswiet (15) uses different numbers for them.

Before entering into any discussion of their relative merit, the author will give a condensed form of Jeswiet's (15) original descriptions. In the following list, the groups are arranged not in numerical order but rather according to their distribution on sides, wing, and juncture of the prophyllum. Diagrammatic sketches, showing the regional distribution of these hair groups are given in figure 4.

Jeswiet and his associates (5, 6, 11, 30), who used the hair groups in describing numerous cane varieties, believed them to be of great taxonomic value. De Calvino (13), on the other hand, considered the hair-group method of classification as an auxiliary method and of use to technically trained persons only. Neither the extreme confidence of Jeswiet nor the equally extreme attitude of De Calvino, which rests mostly on an analysis of conditions pertaining to only one variety (Cristalina), appears justifiable to the writer. To be sure, the usefulness of hair groups in the description and classification of sugarcane varieties is limited, but the method may be effectively used if the hair groups, as they occur, are coordinated with each other and with other characters.

To facilitate description and to make data readily available for comparison, it is often desirable to break up characters into as many fundamental units as possible. Jeswiet must have had this idea in mind when he listed the hair groups on the prophyllum, since their

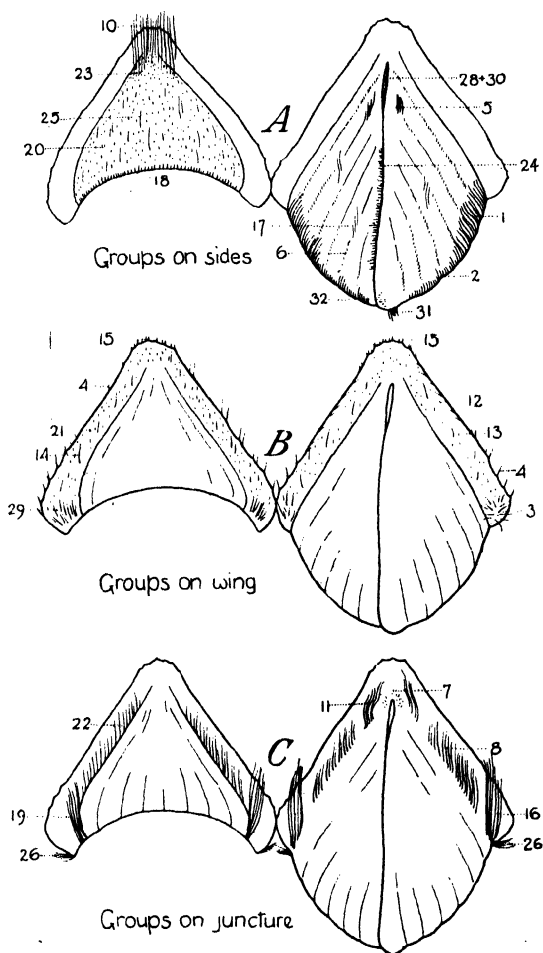


FIGURE 4.—Diagrammatic drawing of prophyllum replicated three times, showing location of hair groups (A) on sides, (B) on wing, and (C) on juncture between sides and wing.

(Legend continued on following page)

(Continued from preceding page)

Hair groups on anterior side (fig. 3, A)

- (1) Lateral groups on overlapping halves of prophyllum. Hairs are white, long, and cover the base of the bud to a greater or smaller extent.
- (2) Basal groups on overlapping halves of prophyllum. These strips of short hairs are often interspersed with groups of longer hairs alternating with the veins and often covering the short hairs.
- (5) Groups of straight or wavy, very appressed white hairs on one or both sides of the germ pore and usually associated with long buds.
- (6) Bands of short brown hairs between the veins.
- (9) A group of long lashes implanted in the germ pore on the inner side of the prophyllum.
- (17) Groups of long, often wavy, appressed white hairs between the veins, giving the side a silky appearance.
- (24) Short lashes along the upper half of the membranous margin.
- (28) Incurved and downward-pointing hairs immediately above the germ pore on the posterior side of the prophyllum.
- (30) Short, inward-pointing lashes on edge of germ pore.
- (31) A group of long, white, downward-pointing hairs implanted at the point of insertion of basal appendage.
- (32) Short brown hairs on the surface of basal appendage.

Hair groups on posterior side (fig. 4, A)

- (10) Group of long hairs implanted on the tip of the side between the veins and possibly extending into region of wing. Hairs may or may not protrude above the bud.
- (18) Strip of long white hairs along basal edge. The hairs may be long or very short, restricted to the center or distributed over entire basal curve. The hairs may form a compact row or small groups.
- (20) Short brown hairs between the veins.
- (23) Short brown hairs on tip, often masked by hairs of group 10.
- (25) Long white hairs between the veins.

Hair groups on anterior wing (fig. 4, B)

- (3) Long, white hairs on base of wing.
- (4) Long, white, oblique or appressed lashes on edge of wing, limited to basal region or occurring over entire edge.
- (12) Small, brownish hairs on surface of wing.
- (13) Long, appressed hairs on surface of wing.
- (15) Lashes at tip of wing; may be considered a part of group 4.

Hair groups on posterior wing (fig. 4, B)

- (14) Long, appressed, white hairs on surface of wing; rarely found in *Saccharum officinarum*.
- (21) Short, brown hairs on surface of wing.
- (29) Long, white hairs on base of wing. Groups 4 and 15 shared with anterior of wing.

Hair groups on anterior juncture (fig. 4, C)

- (7) Groups of short, brown, or long, white hairs above central germ pore.
- (8) Long hairs appressed in regard to the wing or projecting above the wing.
- (11) Long, wavy, white, appressed or protruding hairs on juncture of wing just beneath the wing tip.
- (16) Groups of long white hairs implanted on a broad base in the basal depression between sides and wing.
- (26) Long lashes in corners of the wing.

Hair groups on posterior juncture (fig. 4, C)

- (19) Groups of long, white, and appressed hairs inserted in the basal corners and sometimes protruding above wing.
- (22) A narrow band of appressed, long hairs, often forming a connection between groups 19 and 10. Group 26 is shared with anterior juncture.

number appears to be far larger than a conservative estimate based on fundamental types and regional distribution would warrant. A list of these small units, each designated simply by its number, should suffice when writing up descriptions. Jeswiet not only lists but describes the hair groups for each single variety.

Certain hair groups, as for example group 31, are found only in one restricted location. Others overlap, as for example groups 3 and 13, while some are definitely compound in that they include hairs from different groups.

Groups 1 and 2, fringing the basal part of the prophyllum below the insertion of the wing, are fundamentally one. The fact that the hairs of group 2 are at times short and straight instead of long and wavy is not important, because of the existence of many transitional types. Groups 5 and 17 are also fundamentally alike. Although group 5 is supposed to be located near the germ pore, Jeswiet's own illustrations often show the two groups intermixed. Groups 20 and 23 should be united, since the only difference between them is the restricted location of group 23. Groups 24 and 30 belong together, as already suggested by Jeswiet, and so, for similar reasons, do groups 4 and 15. Groups 3 and 13, on the anterior wing, and groups 29 and 14, on the posterior wing, are fundamentally the same, groups 3 and 29 being the basal parts of groups 13 and 14 respectively.

In studying the buds of a large and varied collection, one comes across forms where the natural regional grouping of hairs does not conform to the theoretical plan. Often the hairs of groups 3, 16, and 26, found on the basal part of wing and juncture, are grouped so closely as to form a single unit (fig. 5). The same arrangement often is found also in the corresponding groups, 19, 29, and 26, on the posterior side. Groups 11 and 10 are usually difficult to delimit. Group 11 very frequently contains elements of groups 13 and 17 (fig. 5); indeed, Jeswiet includes group 11 in group 13. This, however, may not always be done justly, since hairs of group 17 are frequently a component part of group 11. Group 10 shows probably the greatest amount of variation. There are some buds where group 10 is definitely implanted in the tip of the prophyllum. Often when the group is large, elements of group 25 are undoubtedly added; or, if the group is broad, elements of group 14 are included. Jeswiet refers to the fact that no equivalent for group 11 is found on the posterior side. Since in certain buds group 10 appears to be made up of two halves, it is possible that some elements of the two halves are the missing group.

The status of the hairs in the germ pore, groups 9, 28, and 30, does not seem very clear. Group 30 should be considered as belonging to 24, as already suggested by Jeswiet. Group 9, as described by Jeswiet, has never been observed by the writer. Not infrequently, however, long hairs have been seen issuing from the germ pore, but these hairs always belonged to the anterior part of an enclosed leaf. Group 28 is supposed to be inserted in the hollow of the prophyllum just above the germ pore and may be just a modification of group 30, i. e., a part of group 24, which is the general designation for hairs fringing the membranous border of the overlapping half of the anterior side.

In a number of varieties the overlapping margin near the germ pore is covered with long, dense hair (fig. 5). These hairs may be a continuation of group 24, but they differ from that group not only

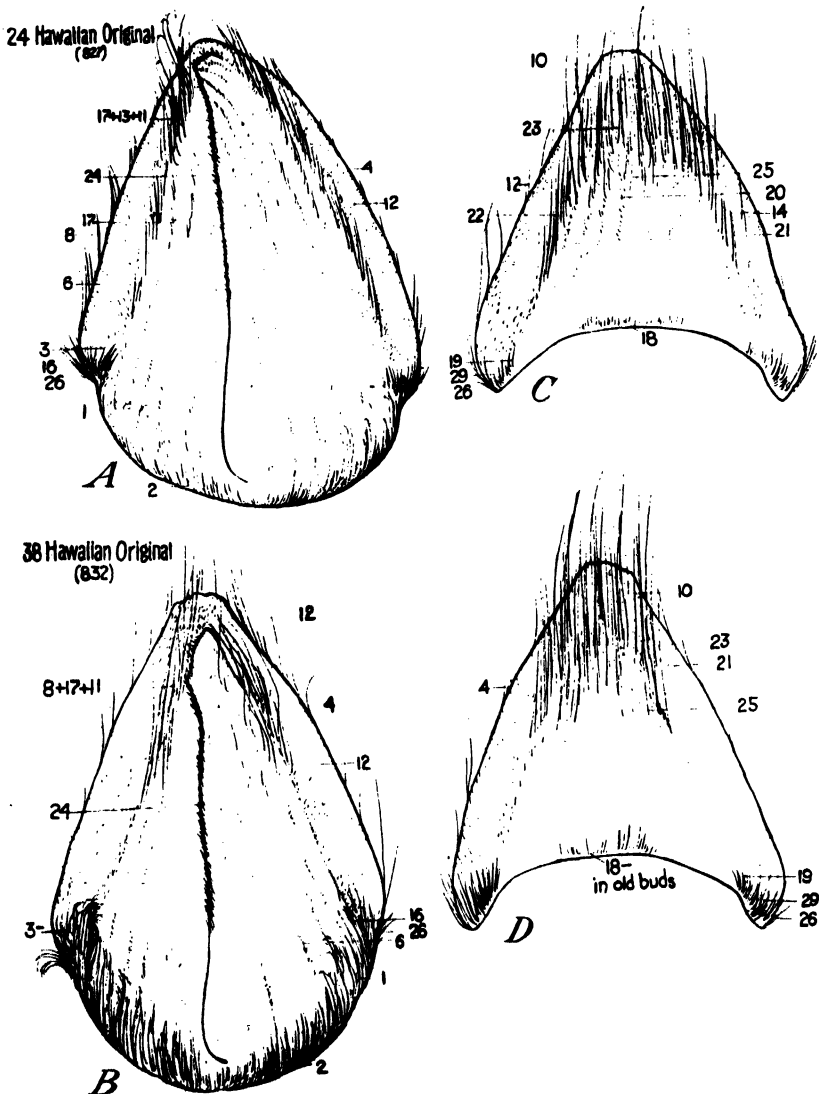
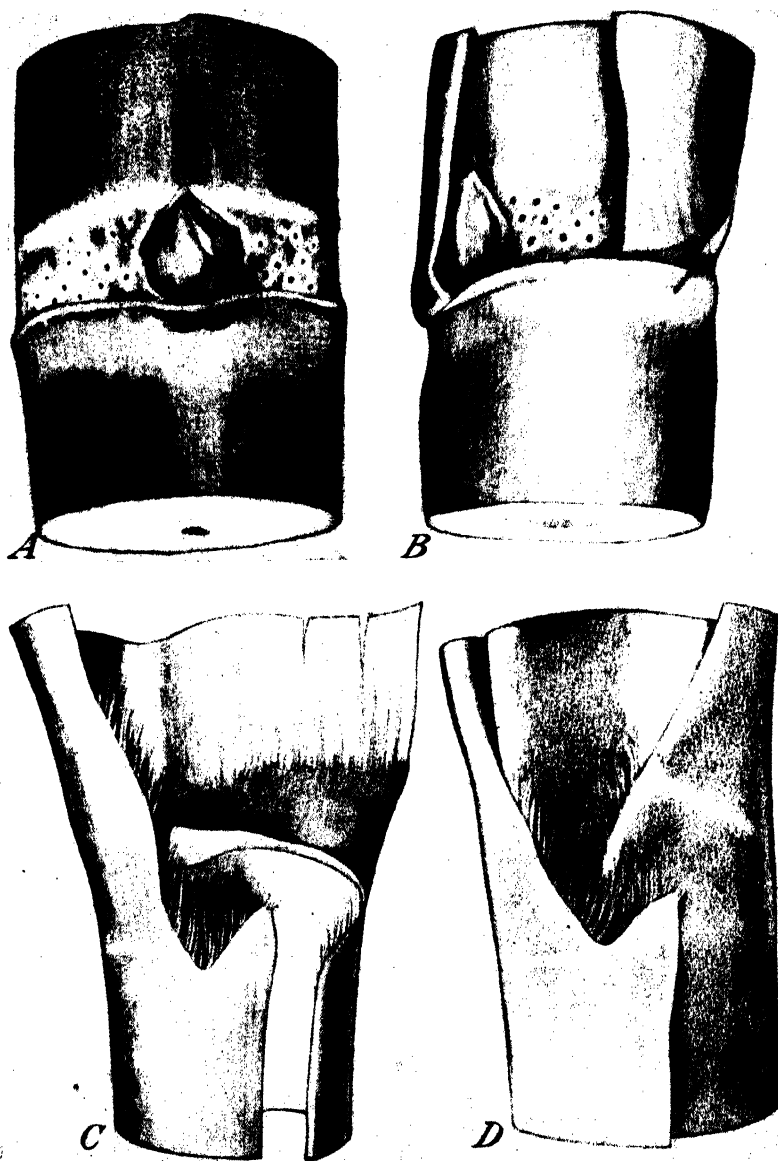


FIGURE 5.—Buds of two Hawaiian Original varieties with typical hair groups on anterior (A, B) and posterior (C, D) sides of prophyllum. $\times 6$.

by their greater length but also by their distribution, not being limited to the margin but covering a prominent band near the margin. They do not seem to fit any of the known groups and should be given a separate number unless the limitation of group 24 is less restricted.



A, Structure of nodal region. Bud inserted at scar; growth ring narrow. *B*, Structure of nodal region with sheath partly cut away to show relation of parts. *C*, Structure of blade joint with ligule. *D*, Structure of blade joint in natural position; outer auricle small and deltoid. All $\times 1\frac{1}{2}$.

MORPHOLOGY OF THE LEAF

In general appearance, the leaves of sugarcane are like those of other grasses. They are borne on the culm in two ranks, one at each node. The number of leaves on a stalk varies with the variety. As new leaves progressively unfold, old ones are shed, so that the number remains approximately constant throughout the vegetative life of a stalk.

Each leaf consists of sheath and blade. At the juncture of sheath and blade is the blade joint with its specialized regions (pl. 6, *C, D*); while at the base of the sheath, where the leaf joins the stalk, there is a distinct swelling—the leaf sheath node or sheath base (pl. 6, *B*).

LEAF BLADE

GENERAL MORPHOLOGY

Leaves when immature are usually stiff and erect. They may retain this position on maturing except that the tips may droop. Most often the blades are ascending; in some varieties, they spread fanlike in a gentle curve.

The blades may be long and narrow or relatively broad, tapering toward the apex, often ending in a long, fine point. There are canes in which the lamina is regionally reduced to the width of the midrib. In these varieties the blade is narrowest where it joins the sheath; it gradually broadens and tapers again toward the apex. Many leaves are relatively broad at the base; they retain this width to a point just below the middle of the blade; from there on the width gradually decreases upward toward the tip. Length and width vary greatly and do not constitute a reliable character. But the ratio of length to width was found to be constant by Panje (29) for varieties of *Saccharum spontaneum*, and this relationship probably holds true for other groups.

The color of the blade is commonly a medium green, parts that are much exposed to the sun showing a reddish cast. In some varieties, especially in the juvenile stage, the color is decidedly reddish or purplish. If much wax is present, the lower surface appears bluish. In varieties with Chunnee blood, according to Jeswiet (15), the dark-green surface of the blade is usually dotted with yellow patches. There are canes in which both the upper and the lower surfaces are covered with a pronounced velvety pubescence; in these, the color of the blade is gray, often with a reddish cast.

The blade is divided by the midrib into halves that are asymmetrical, since the part of the blade that corresponds to the underlying part of the sheath is usually broader than the other half. The midrib is usually prominent. In some varieties it is thick and heavy, in others flat and inconspicuous. Its lower surface is usually dark green, in some varieties marked with a lighter stripe that runs along the center. In the varieties of *Saccharum spontaneum* the midrib is usually white on the upper surface, or lighter green than the lamina. The upper surface of the midrib is channeled, while the lower side is convex near the base. The midrib is always widest at the base and gradually tapers toward the apex; at times, it fades altogether before reaching the tip of the lamina.

The veins run parallel to the midrib. They are of different thickness, a fact apparent to the naked eye in some varieties but more

readily recognized with the aid of a microscope in others. In some leaves the lamina looks conspicuously ribbed; in others, not even the larger primary veins project above the leaf surface. The structure of the veins and its relation to the epidermal pattern will be discussed below.

Although little information concerning texture of leaves is to be found in varietal descriptions of sugarcane, there exists much variation between different varieties. Even to the casual observer, some leaves appear thin, others heavy; some feel smooth, others rough to the touch; some leaves appear leathery, others relatively succulent.

PUBESCENCE

The microscopic pubescence of the leaf will be described in detail in the discussion of the structure of the leaf epidermis. In addition to this, there are two hair groups, designated by Jeswiet as groups 53 and 67, which are readily visible to the naked eye. Group 53 (fig. 6, *E*) is always present, though not always prominent, while group 67 is relatively rare.

The hairs constituting group 67 are found in the intercostal region

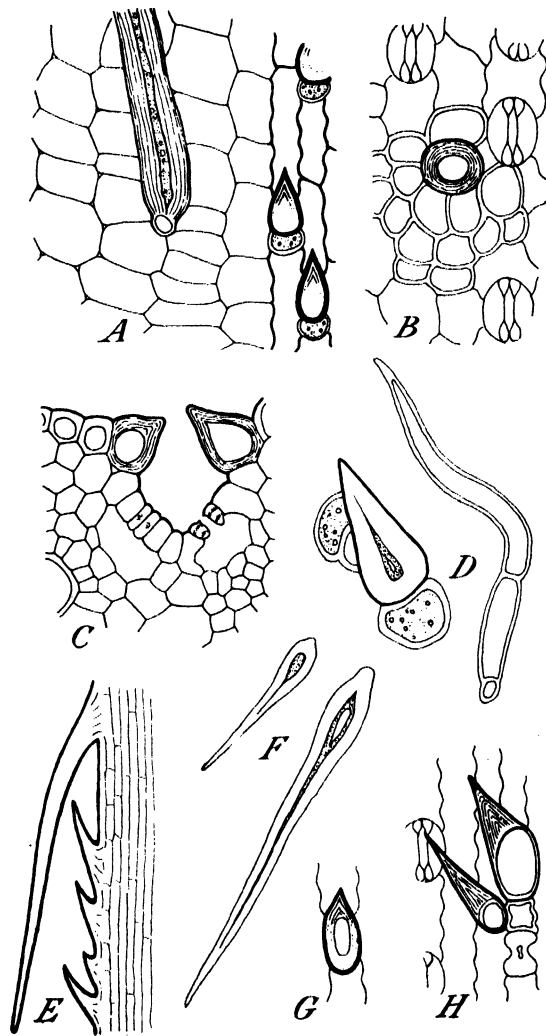


FIGURE 6.—Hairs and spines on leaf and sheath: *A*, Surface view of bulliform zone of the upper epidermis of the blade; in the center is shown the base of a velvety hair (group 67); at the right, cells from marginal zone with short spines. $\times 300$. *B*, Same as *A* but taken from lower epidermis; cells surrounding base of hair are very thick-walled. $\times 300$. *C*, Stomatal groove in lower epidermis of *Saccharum spontaneum*. $\times 480$. *D*, Short spine and two-celled hair from leaf sheath. *E*, Spines from edge of leaf blade. *F*, One-celled hairs from dewlaps. *G*, Solitary spine from midrib region of lower epidermis of blade. *H*, Clustered spines from the lower epidermis of the blade.

of the lower surface and in the zone of bulliform cells of the upper surface (fig. 6, *A*). They are relatively large, long, and soft, as in 28 N. G. 38, Imp. 477, or, occasionally, very stiff. The base of these hairs is raised and is composed of more or less concentrically arranged cells. The walls of these cells are thicker and more heavily silicified than those of the neighboring tissue (fig. 6, *B*). The hairs are always most abundant in the region just above the dewlaps, and denser near the midrib than at the leaf margin. The margin itself is protected by stiff ascending bristles (group 53). In the region just above the blade joint, these hairs are long and relatively soft; higher up, they are short and stiff and often form double rows.

EPIDERMAL STRUCTURE

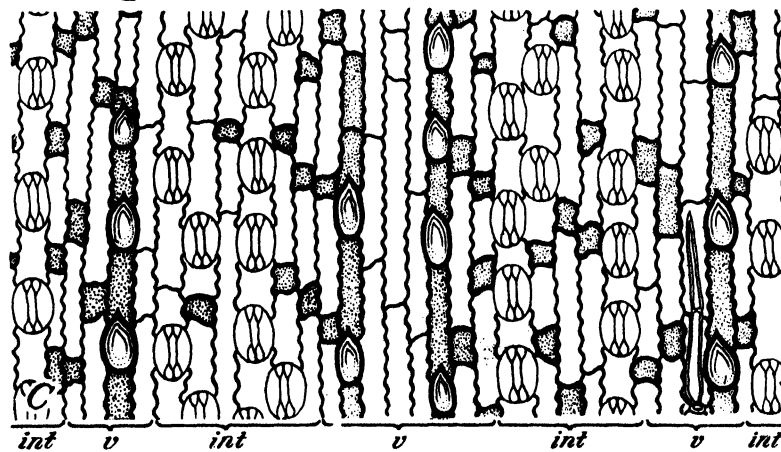
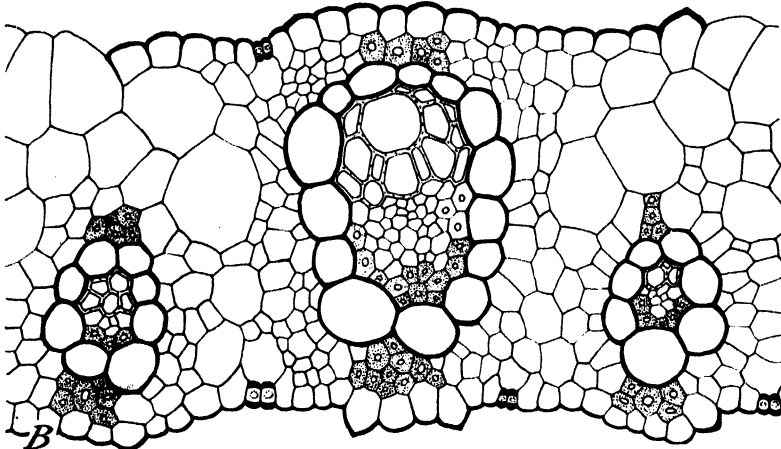
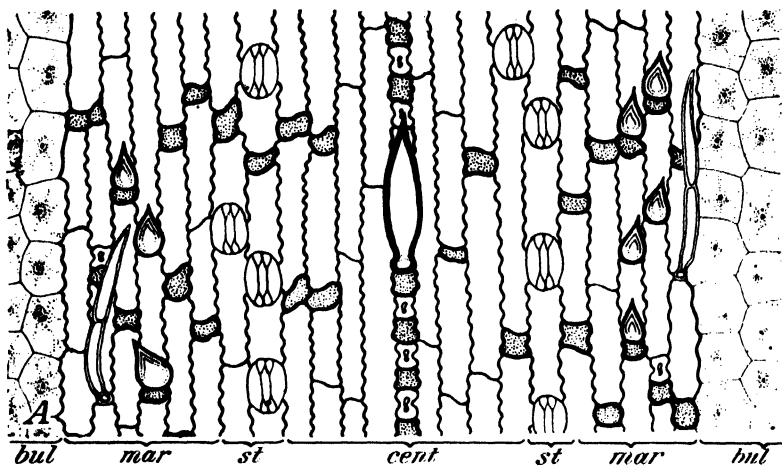
The elements of the leaf epidermis are essentially those of the stem, except that they are modified so as to adapt them to the function of assimilation and protection. In both stem and leaf we recognize a definite epidermal pattern, but, while the stem structure is homogeneous throughout, the leaf shows a periodically recurring design that is related to the tissue elements underlying the epidermis.

CROSS SECTION OF LEAF

An understanding of the inner leaf structure is desirable for the recognition of this relationship; to facilitate it, a leaf cross section with the corresponding lower and upper epidermis in face view is given in figure 7. It will be seen from a study of the cross section that groups of epidermal cells alternately abut on sheath cells and parenchyma cells and that the upper leaf surface has, in addition, bands of bulliform elements usually several cells wide. The cross section shows further that there are bundles of different sizes that alternate with one another. Bundles larger than those shown in figure 7 occur at greater intervals and these alternate with still larger bundles (pl. 7, *A*, *B*). The latter form the primary veins of the leaf and are readily seen with the naked eye, being thicker than the intercostal area and projecting as more or less prominent ridges. Altogether, there are veins of three or four different magnitudes, occurring at regular intervals. All the veins are structurally similar; all except the smallest have two sclerenchyma caps abutting on the lower and upper epidermis. The xylem cap of the smallest veins does not reach up to the upper epidermis but borders on a group of bulliform cells of which the outermost are an integral part of the epidermal structure.

LOWER EPIDERMIS

The surface view of the lower epidermis of the blade (fig. 7, *C*) shows longitudinally running bands of cells of different designs alternating with one another. The position of these bands, as seen in the adjacent cross section (*B*), shows a definite relationship to the type of cells underlying them.



(See legend on opposite page)

Most conspicuous are the bands containing stomata. They are approximately three rows wide and consist of stomata alternating with long cells. At times, the rows of stomata are completely or partly separated from one another by a row of long cells alternating with groups of short cells.

The stomatal bands are flanked by strips of long, narrow cells that overlie the sclerenchymatous bundle caps. As the bundles, which alternate with one another, vary in magnitude, the number of rows constituting the tissue between stomatal bands differs accordingly. The narrowest bands are made up of just a few rows of elongated cells that may be separated from one another by cork cells or cork and silica cells. Bands overlying the large primary veins are much broader. The middle row of such a band and sometimes two adjacent or closely adjoining rows are made up entirely of groups of short cells.

In addition to the cells mentioned, the lower epidermis of the blade has one or several types of hairs. Always present, though in varying numbers, are appressed two-celled hairs, which are most commonly found among the cells bordering the bands of stomata and occasionally scattered among the stomatal rows. Not always present but sometimes exceedingly numerous are short spines (fig. 6, *G, H*), either associated with the two-celled hairs or more usually restricted to the bands of long cells overlying the veins. The spines most often point upward in the direction of the longitudinal axis, but they may be variously oriented. They may be entirely wanting or they may occur in such large numbers as to form one or several uninterrupted rows. Most significant is their presence in certain varieties of *Saccharum spontaneum*, where the stomata are sunken, forming the so-called stomatal grooves (pl. 7, *C, D*, and fig. 6, *C*). Their occurrence has been associated with varietal resistance to mosaic (32), since, wherever they occur, plant lice have no access to the stomata. In this connection, however, it is well to remember that stomata are also found on the upper leaf surface, where they are not similarly protected.

UPPER EPIDERMIS

Except for the added presence of bulliform cells, the elements making up the upper epidermis are the same as those found in the lower though in different proportions. The bulliform cells are easily the most outstanding structural characteristic of the upper epidermis. Depending, as a rule, on the relative size of the veins and their spacing, the bands of bulliform cell rows are wide or narrow, crowded or far apart. The cells themselves often differ with the variety. In some varieties, they are of a honeycomb pattern; in others, they are squarish, somewhat elongated, or broader than long.

The tissue enclosed by the two bands of bulliform cells is for convenience divided into several zones, for which details are given in the composite drawing of cross-section and surface views (fig. 7).

FIGURE 7.—*A*, Surface view of upper epidermis of blade. $\times 320$. *bul*, Bulliform cells; *cent*, central zone; *mar*, marginal zone with two-celled hair and short spines; *st*, stomata. *B*, Cross section of leaf; different types of epidermal cells more or less in juxtaposition with their kind in surface view. $\times 320$. *C*, Surface view of lower epidermis. $\times 320$: *int*, Epidermal tissue overlying intercostal region; *v*, epidermal tissue overlying veins.

Adjacent to the bulliform bands and limited on the other side by one row of stomata is a tissue that may be called the marginal zone. It is from one to five cells wide and contains two-celled hairs and spines. The two-celled hairs are always present, though in varying numbers, and are usually found in the row directly adjacent to the bulliform cells. Spines are absent from some varieties; in others, they may be scarce or abundant. The marginal tissue itself is composed of long cells alternating with short groups that often lack silica cells. Wherever hairs or spines are present, they take the place of silica cells in the short group.

Stomata occur normally in single rows; sometimes their distribution is somewhat staggered, giving the impression of two rows. In some of the wild canes, Shantir Barhni (*Saccharum narenga* Wall.) for example, there are two and even three rows of stomata.

The relatively broad band of tissue between the rows of stomata, the so-called central zone, is composed of elongated cells or long cells alternating with short groups. Frequently the long cells are found in uninterrupted rows, and often the silica cells are wanting from the short groups. The central zone is frequently divided by a narrow band of uninterrupted short cells, composed of cork and silica cells alternating with one another. Sometimes a few of the silica cells are replaced by short spines. These may become very large as compared to the surrounding cells. Such giant spines are frequently found in the upper epidermis in varieties of *Saccharum spontaneum* (fig. 7, A). Where the tissue enclosed between two bands of bulliform cells is rather narrow, the central zone lacks the rows of short cells and the marginal zone is only one or two cells wide.

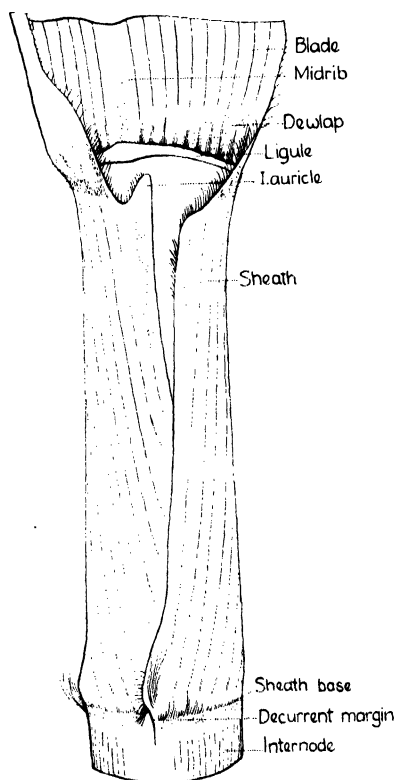


FIGURE 8.—Structure of sheath. To show the parts of the blade joint, the sheath is drawn without the part of the stem that it normally encloses.

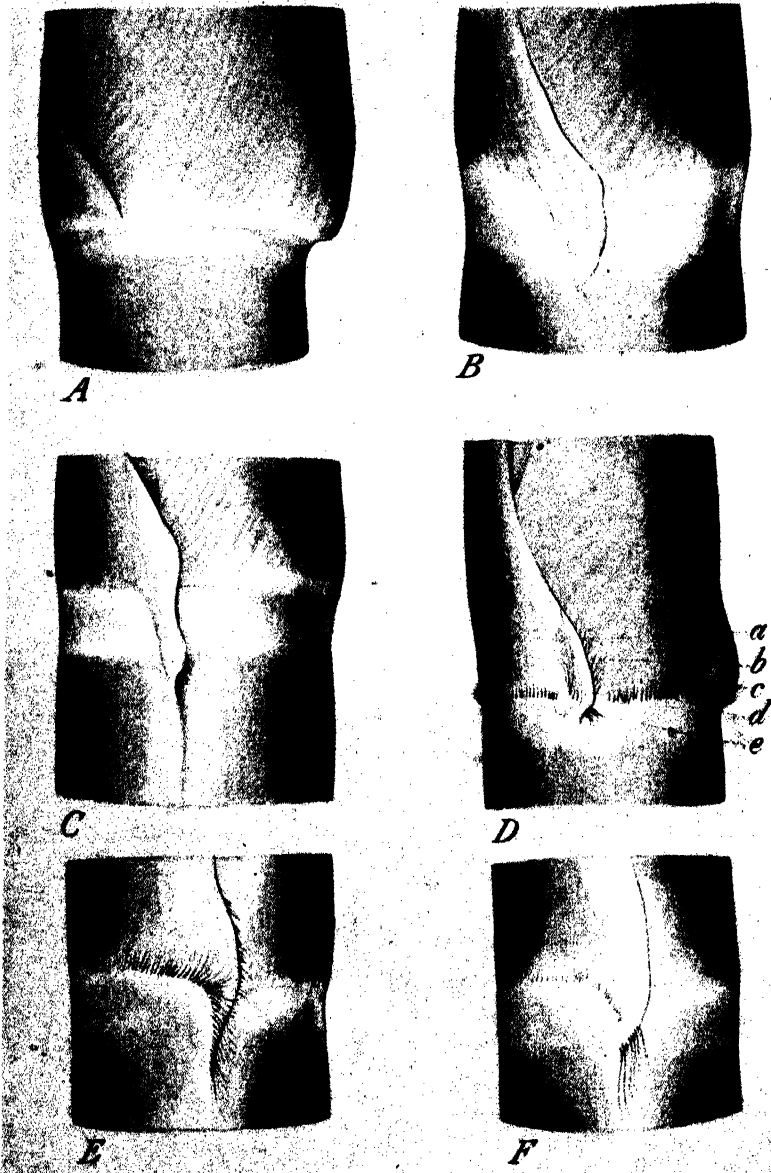
LEAF SHEATH

GENERAL MORPHOLOGY

The leaf sheath envelops the culm above the node and forms an open tube with the margins overlapping (fig. 8), the overlying margin being alternately the right and the left in successive nodes. Flattened out, the sheath represents a rectangular surface, slightly contracted where it joins the lamina below the blade joint and flaring out where it envelops the node (fig. 9). The degree of overlap varies greatly and is not a fixed char-



A, Structure of blade joint of Teboe Glongong (*Erianthus arundinaceus*). Note the narrow leaf blade, the steeply sloping ligule with dense pubescence back of it and pseudo-midrib of sheath below ligule. *B*, Sheath base region of same variety. Tissue of sheath joint darkly discolored. Hair group 59 prominent in sector adjoining overlying sheath margin.



A, Structure of sheath base: Straight, somewhat saccate, and overlying sheath margin not decurrent. *B*, Overlying sheath margin decurrent and appendaged. *C*, Overlying sheath margin prominently decurrent. *D*, Pubescence of sheath base region: *a*, Hair group 64; *b*, hair group 62; *c*, hair group 69; *d*, hair group 59; *e*, separate group, generally considered as belonging to group 64. *E*, Overlying sheath margin decurrent; prominent hair group 64 and partially developed group 59, which in region of sheath margin joins with group 64. *F*, Decurrent and appendaged sheath margin with prominent tuft of hair on lowest tip. This group corresponds to *e* of figure *D*.

acter. Usually the circumference of the leaf sheath at its base is about one and one-third times, in wild varieties up to one and one-half times, that of the stem.

The length of the sheath differs with the variety—from 15 to 52 cm. in the varieties studied—but within varietal limits it is fairly constant.

The sheath is thickest medially and often bulges, saclike, just where it covers the bud (pl. 6, *B*). It is thinner toward the margins, forming near its base a wide or narrow membranous border, which on maturity becomes dry and brown.

The sheath, like the lamina, contains many parallel veins, but these are more widely spaced than in the blade. They are largest in the median part, gradually becoming smaller toward the edges. Usually there is no midrib, although in Teboc Glongong (*Erianthus arundinaceus* (Retz) Jeswiet) the midrib of the blade extends into the upper part of the sheath (pl. 8, *A*).

The sheath is inserted in the tissue of the node, forming an even line around the stalk (pl. 9, *A*). In many varieties the overlying edge of the sheath runs down the stalk, often ending in an inconspicuous or a more or less prominent appendage (pl. 9, *B F*).

The region immediately above the line of insertion of the sheath is called the leaf-sheath node, or sheath base. It is a zone of meristematic tissue, from which the sheath elongates, and is comparable to the growth ring of the stem. Structurally it reflects the molding influence of its location, which demands great elasticity of tissues combined with late maturing. This region possesses increased flexibility, attained by replacement of the sclerenchymatous elements in the vascular bundle sheaths by collenchyma, and also greater strength due to small-celled parenchyma occurring in regions where large air cavities are usually found. When the sheath is fully grown though still young, the sheath base is set off from the rest of the sheath by a differential color, generally yellow orange or yellow green. In varieties that retain their leaves, commonly in wild canes, this color darkens as the leaf ages (pl. 8, *B*).

In canes where the stem cleans itself naturally, the sheath base of old leaves disintegrates, and when a leaf drops off, a scar remains (pl. 6, *A*). In most varieties, the scar is at right angles to the internode though it may be oblique to the axis of the culm, or the leaf scars are

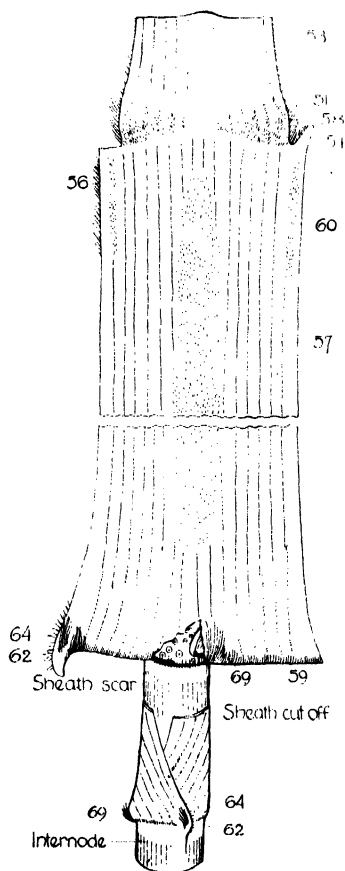


FIGURE 9.—Surface view of sheath, showing location of hair groups.

somewhat oblique in relation to each other. In some forms the scar is even, small, often closely appressed to the stalk on the side opposite the bud, and in others, especially in canes with pronounced saccate sheaths, the scar is squarrose and often sags and spreads under the bud (pl. 4, C).

The color of the leaf sheath is usually light green, sometimes flushed with dark red or purple. The sheaths when young often have a purplish hue; later they may be green. The edges of the sheath are translucent, or they may be reddish. In some striped canes, the color pattern extends into the sheath and, in variegated leaves, even into the lamina. The dark stripes of such canes are usually produced by pigmentation of all layers immediately beneath the epidermis; the white bands are due to regional lack of chlorophyll.

Wax may be deposited uniformly and profusely all over the sheath. Some varieties are only slightly or not at all waxy. Wax deposits vary locally and with age.

The surface of the sheath is smooth or covered with a vestiture of hair. In some canes, hairs are present only on the first-formed sheaths whereas in the later ones the surface is glabrous. The hairs of the unexposed sheaths are much appressed, becoming declinate, especially in the upper region, when the sheath is exposed.

PUBESCENCE

Just as some buds are entirely clothed with hair, some sheaths are also completely covered. Most often, however, the hairs are concentrated in certain regions which have been delimited by Jeswiet (15), who made extensive use of them in his description of cane varieties.

The numbering of the hair groups on the sheath as given by Jeswiet (15) has been retained, but, as in the case of the bud, changes have been suggested and illustrations have been introduced wherever it seemed necessary to clarify the situation.

Jeswiet group 57.—According to Jeswiet, this group is of greatest importance in making determinations. It is found in many cane varieties and it covers the outer surface of the sheath to a greater or lesser degree. In some canes, this dorsal field is very small, is usually inserted high, and consists of only a scanty covering of hairs. Other similarly implanted patches are narrow at the base and then broaden out. Some dorsal fields are implanted low and extend up to the region of the blade joint. In a few canes there is only a small central patch, either linear or lenticular in shape, while in others the field is very irregular and in places so broad as to encroach on the membranous border. The hairs of the dorsal patch may be sharp and long—up to 4 mm.—or relatively short and then usually associated with weakly developed fields. The hairs are almost always inserted obliquely, often appressed but at times almost erect in the upper region of the patch.

Jeswiet group 60.—The two hair groups on the sides of the leaf sheath, the lateral patches, often occur together with the dorsal group, but they are found in fewer varieties. The arrangement on a given sheath is often asymmetrical. Sometimes the lateral field of

the overlying margin is very small, consisting of only a few rows of hairs, while the field on the underlying margin is well developed. The lateral patches are practically always inserted high and usually terminate immediately below the dewlaps. In some varieties, for example Hinds Special, the lateral field of the inner sheath margin extends onto the auricle, a condition which is even more pronounced in some wild canes (Shantir Barhni). Here the pubescence is so dense all over the sheath that no separate groups can be recognized. In variety 28 N. G. 49, the lateral patch broadens just below the collar, forming quite a conspicuous fringe at the basal margin of the dewlaps. Sometimes the lateral patches are rather long, extending over three-fourths of the length of the sheath. They are usually very narrow and remain linear, or they may flare out some distance above their insertion and even partly fuse with the dorsal patch. The hairs of the lateral patches are in length and general character like those of the dorsal patch.

Jeswiet group 56.—This group consists of long or short lashes on the upper part of the sheath margin. In wild canes, where this character is best developed, prominent lashes are found on both margins. The group is wanting in most noble canes and is only weakly developed in some of the hybrids between *Saccharum officinarum* and *S. spontaneum*. Apically the group occasionally abuts on and becomes continuous with the marginal cilia of the auricle (pl. 10, C). In some varieties with pronounced lateral patches in which the hairs crowd up to the margin, the cilia of group 56 become inseparably mixed with hairs of the lateral patch.

A casual examination shows that the region of the base of the sheath is usually smooth, though there are frequently a few small, fairly well-defined hair groups present. Some of these groups belong to the leaf-sheath base. These often stand out most clearly in young leaves, and sometimes they are conspicuous after the leaves are shed. Other groups are found on the surface and margin of the decurrent basal part of the sheath.

According to Jeswiet's description, group 62 consists of silky long hairs covering the lowest tip of the overlying sheath margin, found frequently in seedlings containing Chunnee blood. Group 64 is found in the same region, forming lashes at the lower end of the margin. Jeswiet provides no illustrations for these groups, and his descriptions do not always localize the groups sufficiently for clear diagnosis.

The material examined in the course of these studies shows group 62 as a rather inconspicuous accumbent patch of fairly long, soft, sometimes feltlike hair (pl. 9, D). The basal part of the decurrent margin is occasionally ciliate, and the cilia are easily recognized as group 64. Frequently, however, a third group of downward-declinate cilia is seen immediately above the insertion of the basal appendage. Jeswiet refers to this corner group in some of his descriptions but includes it, as the case may be, sometimes under group 64, sometimes under group 62. Because of its frequent and localized occurrence it should, perhaps, be given a separate number.

Some cane varieties often show a distinct pubescence on the sheath base. The hairs are short, medium long, or very long, and usually point upward, being either appressed or slightly ascending. The

hairs may form a complete or a broken circle. Often they are most numerous and long in that part of the circle which overlies the bud; because of this regional prominence, Jeswiet treats them as a separate group under No. 69. However, on the opposite side of the bud, in the immediate vicinity of the overlying decurrent sheath margin, a similar concentration of hairs occurs in some varieties (especially conspicuous in Teboe Glongong, pl. 10, *B*), which bears no separate number but is treated under group 59. For the sake of consistency, this tuft should also bear a separate number, or else there should be no division at all made in the original group.

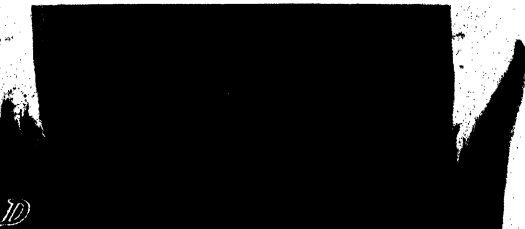
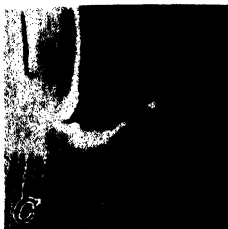
Sometimes the hairs found in the intercostal region of the sheath base, which will be mentioned again in the study of the epidermis of the sheath, are especially prominent, simulating a weakly developed group 59. Sometimes the hairs are short and black and extend lower down into the wax band of the internode below. In canes where this condition is pronounced, it constitutes a valuable character.

EPIDERMAL STRUCTURE

The epidermis of the sheath, like that of the blade, overlies two specialized regions: intercostal area and vein tissue. The intercostal areas of the sheath are much broader than the corresponding areas in the lamina, and the epidermal pattern is much more regular.

The epidermis overlying the intercostal region consists of regular long cells alternating with groups of short cells (fig. 10, *A*). Hairs are of frequent occurrence. The two-celled type (fig. 6, *D*) is always present, though in varying numbers, being most abundant in the region above the sheath base and sparsest in the apical region. Different varieties differ as to the relative distribution of these hairs. In some, the hairs are few; in others, they form a feltlike covering. Short solitary spines are also often found in combination with groups of short cells (fig. 6, *D*) or occurring singly between two long cells. In addition to the short spines, there are relatively large spines present, making up groups 57 and 60, which have been described in an earlier section.

The epidermis overlying the vein tissue is separated from the intercostal region by a single row of stomata which may be locally staggered, giving the appearance of two rows. The epidermal tissue of the vein region may consist of either one or two types of cell rows: rows made up of very long cells (fig. 10, *B*) which may alternate with short groups, or rows made up entirely of cork and silica cells (fig. 10, *C*). One or the other type predominates, depending on the variety. In some canes, there is a large number of adjacent rows composed of short cells only; in others, a few long cells are interpolated within the rows. Frequently bands consisting of rows of short cells are separated by narrow bands or solitary rows of long cells. In some cane varieties the entire vein area is overlaid by long cells, while in others there are bands of only short cells, often bearing solitary declinate spines. The occurrence of spines in this zone is a varietal character. The spines may be few in number and scattered or rather numerous and then in more or less broken rows. They point occasionally in the direction of the vertical axis, though more often they are obliquely declinate.



Structure of ligule and auricles. Color values are reversed, since photographs were taken directly on paper. A, Small outer auricle of Chunnee, showing prominent venation and long cilia on upper free margin (hair group 54). $\times 3$. B, Symmetrical crescent-shaped ligule, rather tall and not sloping; both auricles inserted high and on the same level. $\times 1\frac{1}{2}$. C, Small deltoid auricle with ciliate free margin. Projecting hairs on outer surface belong to groups 56 and 60. $\times 3$. D, Arcuate type of ligule. Inner auricle large and lanceolate, outer one small and deltoid. $\times 1\frac{1}{2}$. E, Inner surface of dewlap with well-developed hair group 52 and inconspicuously developed group 51; auricle very small and of transitional type. $\times 1\frac{1}{2}$. F, Flank of ligule steeply decurrent; auricle absent. $\times 3$. G, Long cilia (group 51) on outer margin of ligule of Kassoor cane. $\times 5$.

There is a slight consistency in the distribution of certain elements of the epidermis of stem, sheath, and lamina.

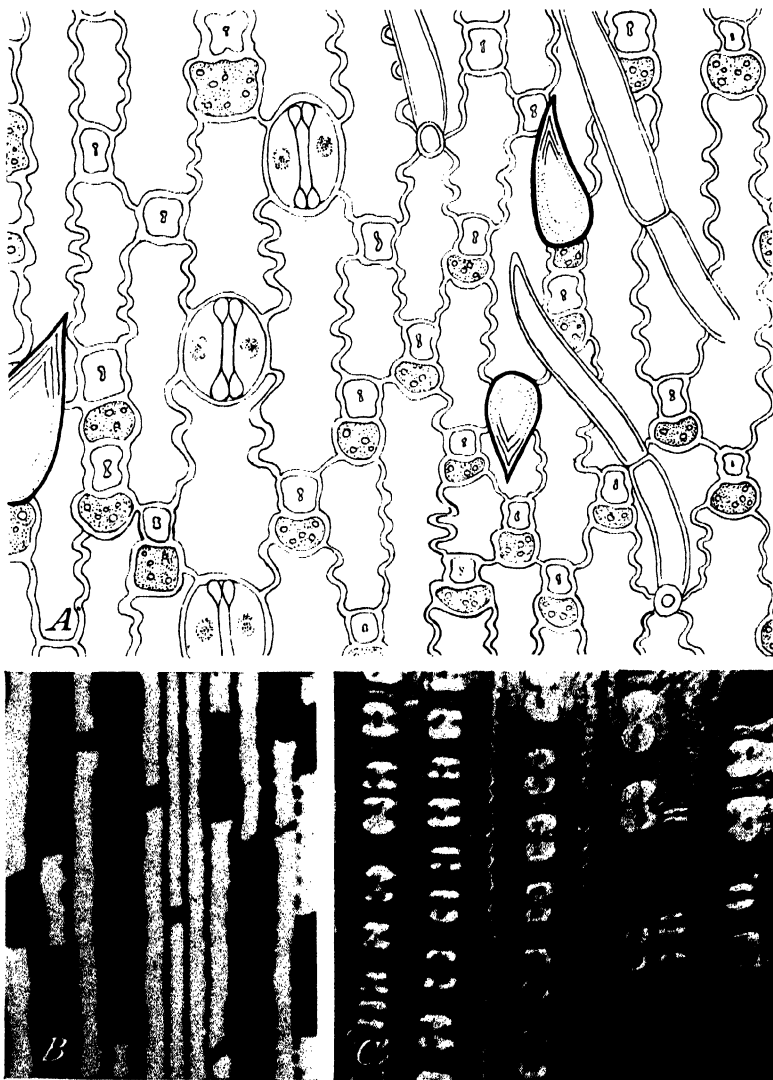


FIGURE 10.—A, Epidermal tissue overlying intercostal area of sheath, $\times 500$. B, Tissue from region overlying vein: rows of short cells alternate with rows of very narrow elongated cells, $\times 300$. C, Tissue from region similar to B but from another variety; practically the entire tissue is composed of short cells, $\times 320$.

BLADE JOINT

Blade joint is the name given to that part of the leaf where the lamina joins the sheath (pl. 6, C, D). The inner surface of the blade joint is known as the throat; it is limited basally by a membranous ap-

pendage called the ligule. The region forming the back or outer surface of the blade joint is the collar. It is composed of two somewhat triangular areas that differ in color and texture from the surrounding tissue; these areas are known as the dewlaps, or joint triangles. Projecting from the sheath margin below the blade joint are pointed appendages known as auricles.

DEWLAPS OR JOINT TRIANGLES

GENERAL MORPHOLOGY

The dewlaps, or joint triangles, form the flanges of the blade joint (fig. 11). They are provided with folds which give the leaf mobility. Structurally the vascular tissue in this region resembles that found in the sheath base. As in the latter, the large bundles have much collenchyma while the xylem consists of spiral vessels.

Although the shape of the dewlaps on successive leaves of a stalk, from the youngest to those fully grown, may vary, there is, nevertheless, a prevailing type pattern. For comparison it is best to select leaves that are mature or almost fully grown and in which the dewlaps have not suffered from the effect of adverse environmental conditions by becoming frayed at the edges or even torn. Also, because of a pronounced asymmetry in the structure of most leaves, only related sides should be compared.

Dewlaps belonging to the youngest leaves are usually of a different color from those of mature organs, though sometimes the color difference is one of degree rather than of kind. Many dewlaps when young are at first a bright red; when mature, a bronze brown. Others when young are yellow; later, a shade of olive. Some are at first grass green, turning to yellow green or olive on maturing. In some varieties, the color of mature dewlaps is brownish or almost black, often modified by the presence of wax or hair. The dewlaps are usually colored uniformly but in some varieties they are prominently edged with yellow or a tint of red.

The outer surface of the dewlaps is more or less heavily waxed, the inner surface less so. The amount of wax deposit varies from a faint bloom to a heavy layer that often hides the natural color or greatly alters it through discoloration upon aging.

In the narrow-leaved wild canes, the dewlaps are always triangular, the apices of the triangles almost meeting on the back surface of the midrib. There is a great variety of forms in the broad-leaved noble canes, but these may be readily assigned to one of three basic groups—the rectangular type, the deltoid or triangular type, and the ligular type (fig. 11, A-I). There are many intermediary forms that tend to bridge the difference between the three patterns.

The shape of the dewlaps is influenced by the slope of the ligule, which marks their lower boundary. In practically all young dewlaps and in many mature ones, the base runs horizontally either in a straight or curved line (fig. 11, I); it may also be more or less undulate. It may have the same outline as the lower margin (fig. 11, I), or there may be a depression in the upper margin in juxtaposition with an elevation in the lower. The outer dewlap margin extends between blade and sheath, and its curve influences the shape of the dewlap. In some varieties, the blade and dewlap margin forms a straight

vertical line; in others, the dewlap has a slight bulge or a depression (fig. 11, *H*) often just above the termination of the ligule and most pronounced on the dewlap corresponding to the overlying sheath margin.

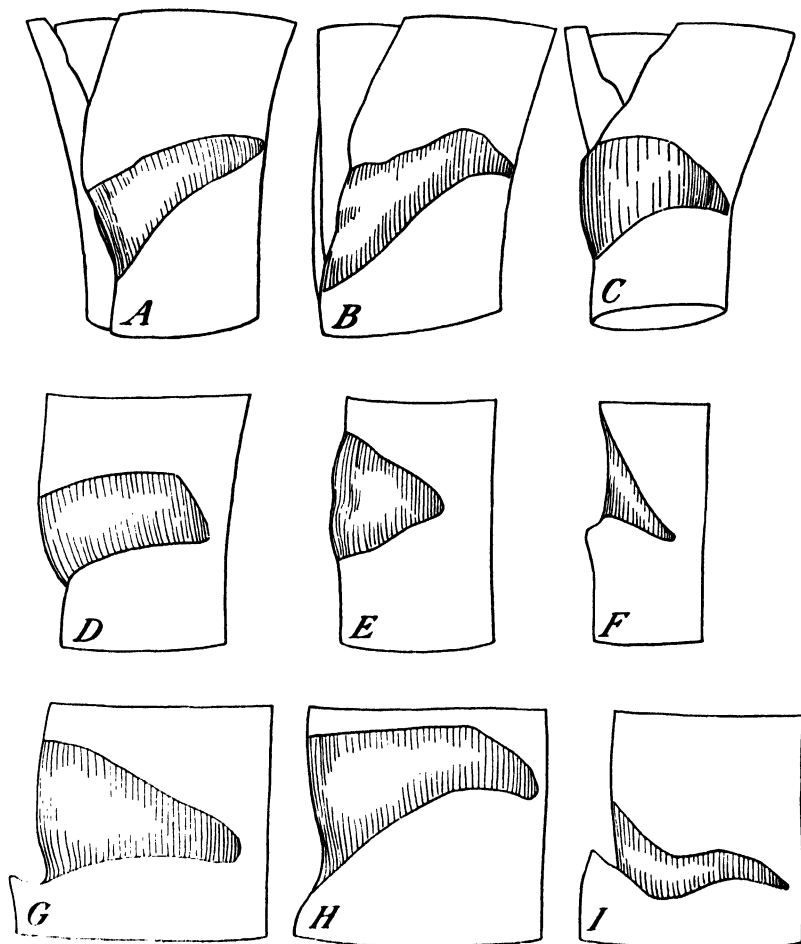


FIGURE 11.—Types of dewlaps: *A*, Very sloping, narrow triangular-ligulate dewlap, Hawaiian Original 24; *B*, very sloping, more or less ligulate dewlap, Louisiana Striped; *C*, tall triangular dewlap with convex upper and lower margin, Rayada; *D*, squarish type of dewlap, Tekcha; *E*, typical deltoid dewlap, Hatooni; *F*, triangular dewlap of the type found in varieties of *Saccharum spontaneum*; *G*, triangular dewlap with horizontal basal margin; *H*, more or less triangular, sloping dewlap with horizontal upper margin, Striped Mauritius; *I*, typical ligulate dewlap, very narrow and practically horizontal, 28 N. G. 251.

PUBESCENCE

The dewlaps have a sparse, dense, or even feltlike pubescence, which is almost always more prominent on the inner or dorsal surface than on the outer or ventral side. The degree of hair development is often in inverse ratio to the amount of wax deposit. The

outer surface is usually heavily waxed, while the pubescence is inconspicuous. On the other hand, the inner surface, which rarely has a prominent layer of wax, is usually very hairy.

The prominent venation of blade and sheath is lacking in the dewlap region, yet the distribution of hairs shows a definite banded pattern, especially on the inner surface.

In the designation of the pubescence of the dewlaps, Jeswiet's hair group numbers have been retained. There is no overlapping of groups except for the cilia of the outer dewlap margin in varieties where the marginal zone of the ventral surface has the long type of hair.

Jeswiet group 58.—The outer surface of the dewlap is, as a rule, only moderately covered with hair, and in some varieties it is practically glabrous. The hairs (fig. 6, *F*) are short, unicellular, and, in mature organs, often hidden under a layer of wax. The entire surface may be uniformly covered with hair or the pubescence may be restricted to the marginal zone. In some varieties, the surface has a dense, feltlike covering and the hairs are uniformly short. In other varieties the hairs are longer near the edges, where they form long marginal cilia. A band or tuft of long hairs is found at the base of the dewlaps in New Caledonia 11 (fig. 14, *E*) and in certain wild canes. The hairs are more or less continuous with those of group 60, if this group is present, covering up the shorter hairs of group 58.

Jeswiet groups 51 and 52.—The upper or throat surface of the dewlaps bears two hair groups. In one group the hairs are short and of the type found on the lower surface; in the other, they are long and often cover up the shorter hair. The short hairs constituting group 52 either cover the entire surface of the dewlap (pl. 10, *F*) or are regionally restricted and prominent only along the outer marginal zone. Besides differences in regional distribution, there is much variation in length and denseness of hairs. In some varieties the hairs are very sparse; in others, they occur in prominent bands or form a uniform feltlike covering. The hairs are straight or curly and usually much appressed. The short hairs of this group are partly, sometimes entirely, covered over by a much longer pubescence consisting of silky lashes that continue to the outer edge of the dewlap. The hairs usually diminish in length toward the midrib. Within the group they are scattered or in strips. In some varieties, like Yellow Tip, group 51 is entirely wanting; in many others, especially those of the New Caledonia collection, it is restricted to a narrow marginal band; sometimes it covers one-half or less of the dewlap surface, and in some few varieties, the entire surface, even extending into the midrib. The hairs near the outer edge are always very long and project prominently over the margin. Some of the hairs grow directly on the edge and become continuous with the long and soft cilia of the basal part of the lamina (group 53). When group 51 is restricted to the marginal zone, it often forms a conspicuous tuft. In some varieties the prominence of the tuft is augmented by the presence of hairs on the tip and margin of the auricles.

LIGULE

GENERAL MORPHOLOGY

The lamina is separated from the sheath by the ligule, a membranous appendage that contains no vascular tissue but is made up altogether of elongated parenchyma cells. In its young state it is translucent and hyaline; later it becomes dry, locally discolored, often brokenly indented and, in most wild cane varieties, torn.

As has already been pointed out for the dewlaps, the asymmetry of the lamina leaves its mark on the organs immediately associated with it. In the structure of the ligule, the asymmetrical condition reveals itself in the following departures from the bilateral design:

(1) One side of the ligule is usually wider than the other side, the degree of variation depending on the difference in width of the two sides of the leaf lamina.

(2) One side of the ligule is often less tall than the other side. This effect is produced by a rapid decrease in height of one side, while the other side retains its maximum height for some distance and then tapers gradually or abruptly as it approaches the edge.

(3) The two sides of the ligule often have a different steepness of slope (fig. 12, *E*), the side which corresponds to the overlying half of the sheath margin having a greater pitch than the other side.

Fundamentally there are four ligular patterns (fig. 12, *A-D*): Strap-shaped, deltoid, crescent-shaped, and bow-shaped.

The deltoid ligule (fig. 12, *B*) is found in many wild canes and is associated with the narrow type of leaf, especially with varieties where the lamina has been reduced to the width of the midrib. In this type of ligule, the height is about equal to the width at the base. In all other forms, the width at the base is many times the maximum height.

In the strap-shaped ligule (fig. 12, *A*), a relatively rare type (Raiatea 1, Imp. 923), the height remains practically unchanged. The crescent- and bow-shaped ligules are tallest in the middle, gradually or rapidly thinning out toward the margin of the leaf. In the crescent-shaped type, there are forms with a relatively high and convex central part and forms in which the upper free margin remains horizontal (pl. 10, *B*). In the bow-shaped type, the central part is depressed with the flanges either horizontal or sloping. The upper free edge of the ligule may be entire or notched. The latter condition is often restricted to the flanges near the margin of the leaf; however, the entire edge may be emarginate. The notched or emarginate condition of the ligule must not be confused with structural changes accompanying the aging of this organ as is observed especially in the deltoid type, which on maturity often becomes torn.

The middle part of the ligule, which overlies the midrib, varies in height between 2 and 8 mm. It shows the greatest variation in shape and generally is responsible for the many different and intergrading types. The upper margin of this region is flat, raised, or depressed, producing the broadly curved, pointed, or arcuate ligular effects. A depressed upper central region is always associated with the bow-shaped ligule (pl. 10, *D*), while curved sloping flanges and a horizontal central edge produce the subarcuate types.

The lower margin of the ligule, which is inserted in the tissue of the leaf, also shows a variation in the shape of the central region. Crescent-shaped ligules with a broad- or round-pointed upper-central region may have a convex basal margin; in some, this region is obcordate, projecting downward rather than up.

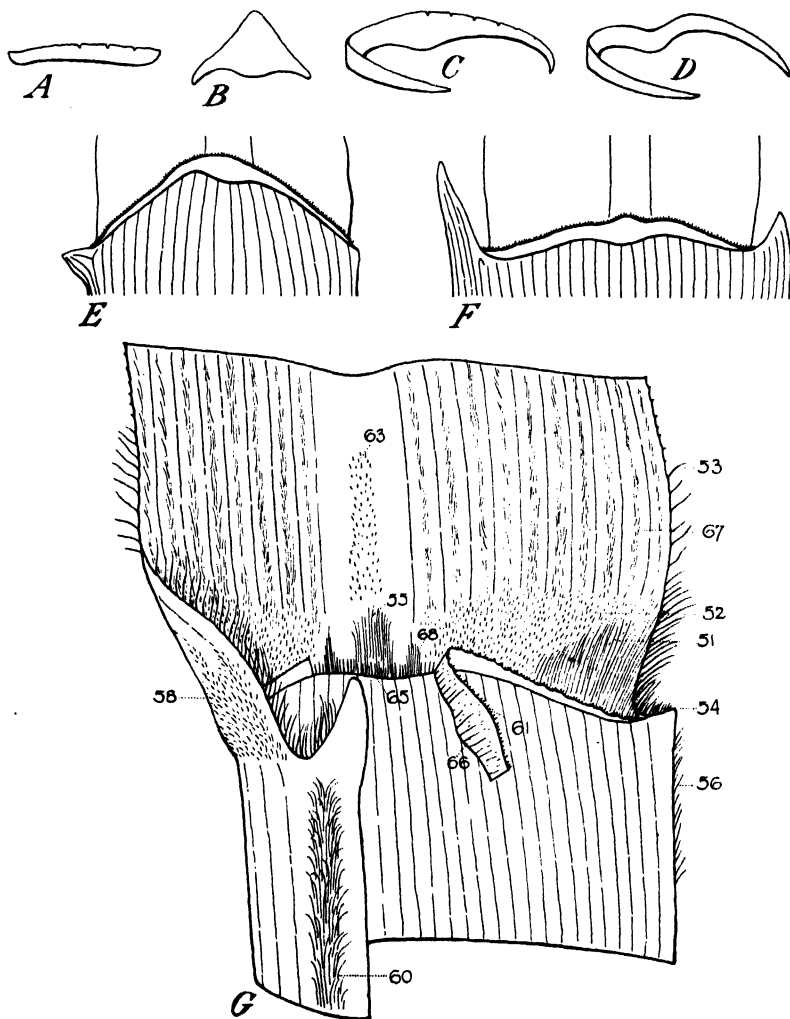


FIGURE 12.—Structure of different types of ligule: *A*, Strap-shaped; *B*, deltoid; *C*, crescent-shaped; *D*, bow-shaped; *E*, asymmetrical, steeply sloping ligule; *F*, asymmetrical horizontal ligule; *G*, detailed structure of blade joint, showing location of the various hair groups.

The two ends, or flanges, of the ligule normally terminate at the edge of the leaf. In some forms with transitional types of auricles, these flanges may project a short distance beyond the leaf edge; in others, they may be abnormally decurrent, running almost vertically downward before terminating (pl. 10, *F*).

PUBESCENCE

The ligule is glabrous and shiny on the adaxial surface. The side next to the leaf is rarely smooth but is usually covered with partly free or completely adnate hairs (Jeswiet group 66). These dorsal hairs arise at the base of the ligule, remain adnate to the epidermis for a certain distance, become free near the apex, and often project some distance above the free margin of the ligule. In certain varieties like Striped Mauritius, the hairs of group 66 are adnate in the center of the ligule but completely free at the flanges and often several times the height of the ligule. Sometimes the dorsal hairs are very few in number; at other times they are dense and the surface may appear slightly ribbed wherever the hairs are completely adnate. In certain varieties the hairs are short; in others, they project quite far above the free margin of the ligule.

The upper margin of the ligule is almost invariably ciliate, although there is much variation in both density and length of these hairs. Sometimes the cilia are inconspicuous in the central part and prominent at the flanges. The hairs making up group 61 (pl. 10, *G*) are really of two types: those that are extensions of the apical epidermal cells and those that morphologically belong to group 66, being the free tips of hairs that originated near the base of the ligule but remained completely adnate, becoming free only a little distance below the upper margin.

PUBESCENCE ON MIDRIB

On the midrib behind the ligule, there are found, in certain varieties, a number of hair groups (figs. 12, *G*, and 13, *A-D*) which Jeswiet named groups 55, 63, 65, and 68. Not all of these groups are found on one and the same stalk, not even in one and the same variety; but a combination of two groups is not uncommon.

Group 55, according to Jeswiet (15), is very rare in *Saccharum spontaneum*, although he refers to it quite frequently in his description of varieties. It was observed by the writer in a number of canes from the New Caledonia and other collections. It is a fairly prominent group of rather long or medium-long hairs, forming a shallow tuft the width of the midrib or narrower. Associated with it, although often occurring independently, is group 63. This group forms a triangular or linear patch, from a few millimeters to several centimeters in length, usually inserted directly at the base of the ligule or, if group 55 is present, directly above this group. The hairs are always short and appressed, scattered or crowded, usually inserted obliquely, and visible to the naked eye because of their silvery sheen.

Group 65 differs from 55 in having the hairs arranged in a single row which does not project above the free margin of the ligule. According to Jeswiet (15), the row extends over the entire width of the leaf blade. But in his varietal descriptions (15-24) he limits it to the region of the midrib, a location which is in agreement with the data embodied in this paper. Group 65 appears to be closely associated with the dorsal pubescence of the ligule (fig. 13, *A*). In varieties in which the hairs of group 66 are long and free, one finds that, upon separation of the ligule from its place of attachment, some of the long hairs remain with the ligule while other hairs belonging to the same group are left behind as a fringe implanted in the tissue of the blade

(fig. 13, A). In varieties where group 55 is small and the hairs are short, it is easily mistaken for group 65. Another difficulty is encountered in canes where the long hairs of group 51 reach as far as the midrib; in such instances, it is impossible to trace group 65 beyond the confines of the midrib. This may have been the reason why Jeswiet in his descriptions limited the group to the midrib.

Of rare occurrence is Jeswiet's group 68—two pointed tufts of long or medium-long hairs implanted behind the ligule on the two

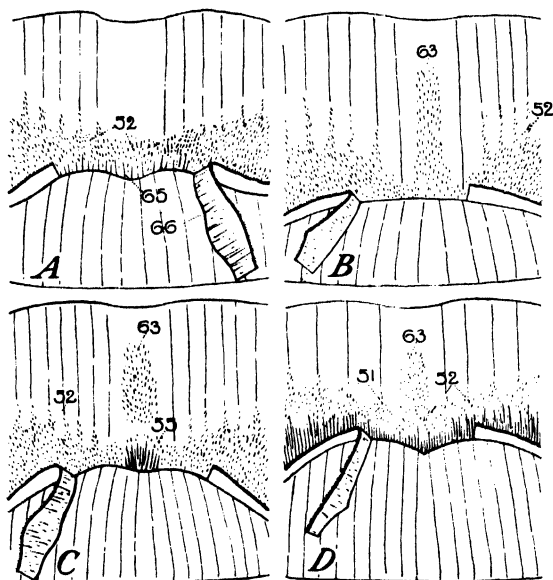


FIGURE 13.—Hair groups on midrib and their relation to the hairs of the dewlaps; the ligule is folded back to show insertion of hair groups. A, Group 52, extending across midrib; hairs of groups 65 and 66, similar in origin and appearance. B, Group 52, extending partly across midrib; group 63, attached to base of ligule. C, Group 52, extending across midrib; group 55, inconspicuous; group 63, fairly prominent but short. D, Hairs of groups 51 and 52, extending across midrib.

outer margins of the midrib and basally connected with group 65. This group has not been observed in the material studied by the writer.

Although groups 51 and 52 usually terminate at the midrib or some distance before reaching it, there are varieties, especially among the wild canes, in which either or both of these groups reach across the midrib, making the identification of the four midrib groups difficult or impossible.

AURICLES

GENERAL MORPHOLOGY

The auricles are ear-shaped appendages of the leaf sheath. In sugarcane they form pointed structures that are asymmetrical, often weakly developed or altogether wanting. Auricles, if present, reach full development rather early in the ontogeny of the leaves; they are well developed, even on the youngest leaves, although

differing in shape from those on mature organs. As soon as the blade joint is exposed, the auricles begin to degenerate; they become dry and brown and often shrivel up, the change keeping pace with similar changes in the upper sheath margin, which, as we have seen earlier, becomes discolored and dry on maturing.

Jeswiet (15) recognizes four types of auricle development, which are briefly as follows:

- (1) Canes with two well-developed auricles but with the inner one the larger.
- (2) Canes in which only the inner auricle is well developed, while the outer one is a transitional structure.
- (3) Both inner and outer auricles of the transitional type.
- (4) Both auricles wanting and the leaf sheath merging imperceptibly with the blade.

This classification has been retained, but, for the sake of convenience, it was found advantageous to subdivide the first group into canes in which both auricles are well developed and canes in which the inner auricle is invariably larger than the outer.

A typical cane variety in which the auricles are altogether wanting is Hatooni; the sheath is of the same width as the blade and only a slight constriction in the dewlap region marks the transition.

Canes with the transitional type of auricle are very common. The blade passes into the sheath, either with a slight curve or rather abruptly under a right angle. The latter type is frequently found in Jeswiet's second class, where only the inner sheath margin has a well-developed auricle. When both auricles are of the transitional type, the outer one may be right-angled while the inner joins the blade under a slight curve. In some varieties, the reverse holds true.

The types with one or two well-developed auricles (fig. 12, *F'*) are most common. A good example of the latter is Red Tip, which has two large auricles, both of approximately the same shape.

The auricles are inserted high or low, at the same level or at different levels. In horizontal ligules, the line of insertion of the two auricles lies in the same plane and, if the auricles are large, their tips may project prominently above the upper margin of the dewlaps. The insertion point of the auricle usually coincides with the termination of the ligule, but in many Hawaiian Originals, which have decidedly sloping ligules, the inner auricle is inserted at a considerable distance below the termination of the ligule (fig. 14, *C*).

The asymmetry of the auricles reveals itself both in the different size of the two organs and in their height of insertion. In forms with horizontal ligules, the auricles are inserted high; in forms with sloping, asymmetrical ligules, the inner auricle is inserted somewhat higher than the outer.

Although there is some variation in size and shape of the auricles on the same stalk, a pattern characteristic of the variety predominates. The most typical auricle shapes are illustrated in figure 14. Most common is the deltoid pattern, varying from the short and rather blunt to the lanceolate type. Auricles of the lanceolate type are very common and are found in almost any size. Less frequent are the falcate, calcariform, and unciform shapes.

PUBESCENCE

The free upper edge of the auricle is smooth or ciliate (Jeswiet group 54). The hairs often cover only part of the free edge, but

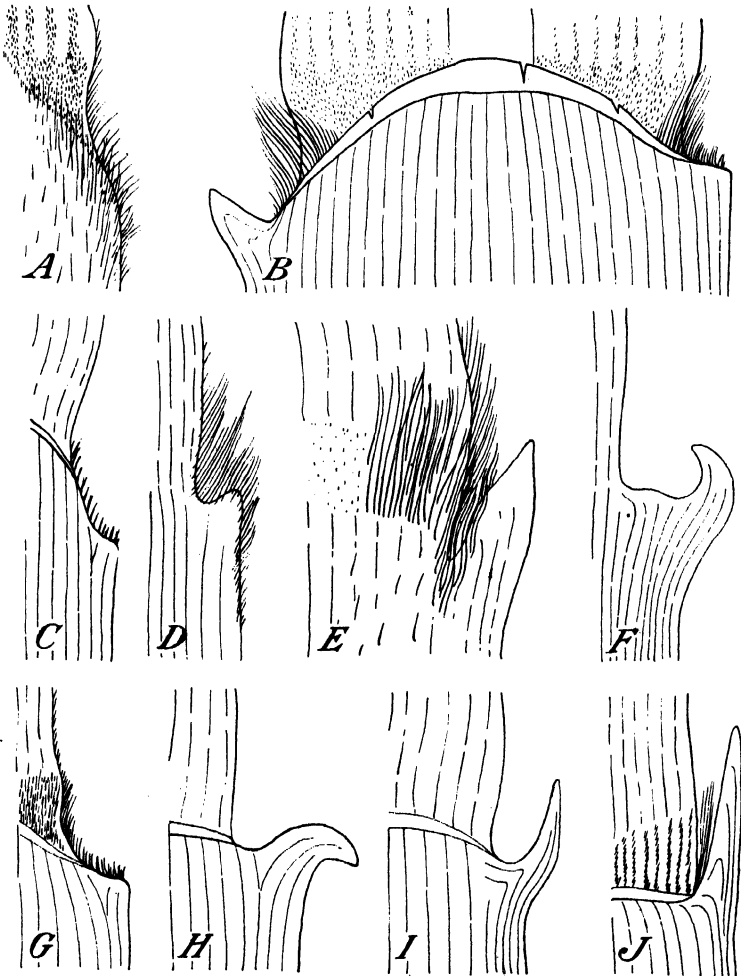


FIGURE 14.—*A*, Long spines of group 60 become confluent with group 54 and partly cover short pubescence of dewlap, 28 N. G. 49; *B*, outer auricle of transitional type, inner auricle deltoid and inserted lower than outer auricle, N. C. 53; *C*, low-inserted inner auricle, Hawaiian Original 36; *D*, hairs at overlying sheath margin (group 60 or 56) and hairs on tip surface of auricle (group 70) confluent with marginal cilia of auricle (group 54), Chunnee; *E*, hairs of group 60 partly cover outer surface of dewlap, N. C. 11; *F*, unciform type of auricle; *G*, transitional type of auricle; *H*, calcariform type of auricle; *I*, falcate type of auricle; *J*, lanceolate type of auricle.

in some instances they extend to the very tip (pl. 10, *A*) or even descend down the outer margin (pl. 10, *C*). In the transitional type of auricle, where the sheath changes into the blade under a right or an obtuse angle, the edge may be ciliate up to the sheath

margin and, if group 56 is also present, the cilia of the two groups may appear continuous with one another.

In a few varieties, the surface of the auricles is covered with long hairs, mostly along the outer margin. When occurring on the inner auricle, the group is known as 71; when on the outer auricle, as group 70. Jeswiet (20) reports them principally for Chunnee crosses. In varieties with strongly developed lateral patches (group 60); hairs of these groups may become continuous with those of the auricles. When the surface of the auricles appears glabrous, the epidermis under the microscope shows nevertheless numerous short spines, which take the place of silica cells in the typical short group.

STABILITY OF CHARACTERS

The less variable a character, the more useful it is in the general classification scheme and in delimiting a variety.

Characters may differ qualitatively and in degree, representing the expression of inherited tendencies modified by environment. Some of the changes take place during the normal course of development; others are speeded up or retarded by abnormal external conditions. Excessive variability may be due to an unbalanced hereditary make-up.

Certain characters, such as pubescence and structure of epidermis, are fixed during early development; others change progressively, attaining full expression at the culmination of vegetative growth, with some modifications even after the advent of maturity. Frequently retrogressive changes set in, resulting in death or the loss of an organ.

Hair groups and epidermal structure are largely fixed characters that can be studied satisfactorily in young plants though some hair groups become more prominent late in ontogeny. Qualitative variations, such as the occasional absence of certain hair groups or their unilateral distribution in some of the buds on a stalk, are quite common; they are apparent very early and may not change in character as the plant continues development. Certain hair groups, like group 58 on the outer side of the dewlaps, are most conspicuous when young, since secondary changes, e. g., the deposition and subsequent discoloration of wax on the dewlaps, are apt to mask the hairs, making evaluation of the group difficult. The epidermis, on the other hand, is best studied in mature material, not because of any change in the epidermal pattern but because of the greater ease of handling mature tissue in preparing it for microscopic examination.

The great majority of characters are modified in one way or another during vegetative growth. Usually only certain aspects of a character are changed while others remain stable. An instance is the ligule, which shows a characteristic type pattern even in very young leaves. Changes concomitant with further development may involve only an increase in slope in varieties that are characterized by a steeply sloping basal region.

During ontogeny, organs not only enlarge but also change in shape. The change may be superficial or pronounced, but almost always there is a distinct resemblance between the juvenile and the mature form.

Practically all internodes are cylindrical when young but assume a characteristic form pattern at or somewhat before maturity. Abnormal environment such as prolonged dry spells may cause, according to

Jeswiet (15), cylindrical internodes to become conical and tumescent internodes to become cylindrical, while other factors are responsible for the appearance of short, tumescent forms. Associated with the development of certain internode patterns is the shape of the root band; obconoidal internodes are joined to conoidal root bands, and internodes shouldered at the base opposite the bud have an obconoidal sector in the corresponding region of the root band. Changes in the growth ring during development are limited. The young growth ring is usually flush with the internode or somewhat depressed; later it may bulge as a narrow ring on the bud side or around the entire circumference. Changes in shape of dewlaps are in the beginning associated with changes in the slope of the ligule and later with a change in leaf carriage. Young dewlaps are often narrow, with a more or less horizontal basal margin. Some retain this form during subsequent growth; others may change this strap-shaped juvenile form to a more characteristic deltoid or squarish type. Often the change is more apparent than real, since an unmistakable type pattern persists during growth.

The situation is somewhat different in auricles that are advanced in development, even in young leaves. They are apt to retain their juvenile form if it is of the conventional deltoid or lanceolate type, but change if of a more unusual design, viz, unciform, calcariform, or falcate. Auricles of the transitional type are alike in young and mature leaves.

Buds usually enlarge uniformly during ontogeny; they may lose their flatness but retain a fundamental design characteristic of the variety.

Color changes affect all parts of the plant that are protected when young and become exposed in the course of development. Often there is merely a darkening or intensification of the color; at other times, the change is more radical, as in certain dewlaps that are bright red when young and brownish olive when mature. Growth ring and root band are at first a pale green or ivory; they change gradually until they are approximately concolorous with the internode. Sheath and leaf blade often become a darker green, and the purplish tinge that characterizes the young state in some varieties is absent from the mature organs.

In a normal environment, these color changes follow a given sequence, so that the color of a cane during vegetative growth is rather typical of a variety. If exposure to light is gradual, the color variations, especially in the stem, are not nearly so pronounced as when the change is abrupt; for this reason a cane from the middle of a field has stalks colored differently from canes at the edge of a planting. The quality of light influences color. This is noticeable in greenhouse specimens, which frequently differ in stem color from a plant of the same variety grown outside. Higher altitude with accompanying lowering of temperature has, according to Jeswiet, also a marked effect on pigmentation, so that the color of a variety growing in the plains differs from that grown at higher elevations.

Sunlight not only affects the color of a stem but may further alter the stem surface by inducing formation of corky cracks or by intensifying their development. Often the color is modified by the presence of wax, with additional changes induced by the growth of molds that

may give the effect of a sooty discoloration. Other changes are effected by the sloughing off of the waxy layer in stripes or irregular patches. Hairs and wax, separately or in combination, often affect the color of the dewlaps. In young organs the felty pubescence often produces a silvery sheen, masking the original pigmentation; later in ontogeny, heavy layers of wax may be deposited, which, on discoloration, give the dewlaps a dirty-olive or blackish tinge, regardless of the original color.

Certain changes of form are coincident with the development of neighboring organs. An instance is the dewlaps, which often have to adapt themselves to a changed leaf carriage. When the leaves change from an erect to an ascending habit the dewlaps compensate at first by enlargement whereby the length of the outer dewlap margin is increased, later by passive stretching, a process facilitated in leaves that have conspicuous folds in the dewlaps. Eventually the dewlaps become torn, especially during unfavorable, stormy weather. Other organs merely enlarge after maturity. Thus flat buds may become plump, bend away from the stalk, and elongate; root primordia swell and develop into rootlets. All these changes represent tendencies that are normal in some varieties but are induced in others by changes in environment favorable for their expression.

Quite common are certain retrogressive changes that may set in even before the advent of maturity. The upper membranous margin on the leaf sheath and also the auricles begin to wither and turn brown before growth is complete. Similar changes take place in the sheath-base region. This part of the leaf sheath, which first acts as an intercalary meristem during active elongation, becomes the zone of abscission when the leaves are shed. If the leaves are retained, the sheath base darkens and shrivels somewhat. The ligule loses its transparency, the margin develops cracks, the texture becomes leathery, and the color an uneven brown. The dewlaps, as already stated, are similarly affected; they become discolored and torn, often long before the leaves are shed. The membranous margin and basal appendage of the prophyllum wither and become discolored while active growth may still be in progress; eventually the entire prophyllum turns an uneven brown. While certain of its hair groups, especially on the wing, tend to become more prominent, those on the side of the prophyllum grow less conspicuous so that old buds have a striking, polished appearance.

Stability of vegetative characters is only relative, for what appears more or less fixed in one variety shows much fluctuation in another; this applies to both qualitative and quantitative characters. For example: Hair group 11, on the anterior side of the prophyllum, may be found on all buds of a stalk or stool and be equally prominent on both halves of the anterior side; in another variety, the group may be limited to a certain number of buds; in a third, the group may show further reduction by being occasionally absent from one or the other half of the anterior side. It goes without saying that hair group 11 has a greater diagnostic value in canes of the first group than of the other two. Other characters may show even less dependability. A normal environment may intensify normal variations or have little or no effect on them. Characters that vary little are usually associated with conservative organs. A structure of ancient origin, without much tendency to variation and not subject to modification under the influ-

ence of environment, is the ligule, as shown by Panje (29) in his study of *Saccharum spontaneum*. The stem epidermis is another structure that is especially useful in tracing out taxonomic relationships.

The proper evaluation of hair groups from the standpoint of their taxonomic usefulness is at best an unsatisfactory undertaking. A glance through Jeswiet's and the author's varietal descriptions shows that only a certain number of hair groups, rarely more than 50 percent, are constant and these include the more common groups; the ones most suitable for diagnostic purposes are often absent and their distribution is usually erratic. It is of little comfort to have on the check list such groups as groups 1, 12, 21, and 26, since their occurrence is almost universal on sugarcane buds; only quantitative and, to a certain extent, qualitative differences within group limits, if constant, give these groups a certain value. Often the mass effect produced by the bud pubescence in general is more important for rapid diagnosis than is a study of the individual groups. Occasionally, certain solitary groups are so prominently developed that their presence suffices to delimit a variety.

The hair groups on blade and leaf sheath are often more reliable and more easily evaluated than those on the bud. Their utilization in descriptive work involves qualitative as well as quantitative differences. Group 57, for example, is either present or wanting but, when present, may vary in distribution so much that it loses its value as a quantitative character. Group 60 occurs relatively seldom and is proportionately more valuable than the dorsal patch. The marginal fringe of the ligule is very useful in extreme cases (e. g., Kassoer, with a very tall marginal fringe, compared to Shamsara, where these hairs are extremely short). On the other hand, the hairs on the midrib back of the ligule often show too much variation to be really dependable, although in certain varieties (N. C. 11, 29, and 78) some of these groups (63 or 55) are rather constant. The hairs on sheath joint and decurrent sheath margin are of importance in certain taxonomic groups. Group 59 is very variable, occurring over the entire circumference of the sheath joint or in localized regions; often a certain regional distribution of hairs is so constant as to constitute a character within a character, making it seem advisable to break up this group into smaller units.

The velvety pubescence of the blade (group 67) is very striking and represents one of the most clear-cut characters. It is not so rare as suggested by Jeswiet and should be of value in any classification scheme.

The microscopic pubescence of leaf blade and sheath, in fact the structure of the epidermis of these organs in general, is often important in differentiating varieties when tracing relationships. For example, stomatal grooves are found on many but not all wild canes, and the giant solitary spines occur on the leaf lamina of only a few of these varieties.

Occasionally certain characters are so limited in their distribution as to have been recorded only once or twice in literature. An example of such a character is the hairiness of the stalk of New Caledonia 32 and 75 Lehu of the collection recently studied by the author.

SUMMARY

Sugarcane is a tall perennial grass with the culms bunched in stools or evenly scattered, erect, ascending, or prostrate. The internodes are alined or staggered and attain their characteristic development—form, length, diameter, and shape of cross section—in the middle part of the stalk; dwarfed and abnormally long internodes may occur. The surface of older internodes is often marked by corky cracks or growth cracks, and there may be a more or less conspicuous bud furrow present. The stalks are covered with a layer of wax of varying thickness that forms a distinct band at the top of each internode. The color of the stalk varies in young and milling cane, and it may be modified by external factors and by the action of surface mold on the wax deposit.

The internal tissue of the stalk has a uniform color, or the center may differ by being whitish and pithy; often there is a distinct cavity in the center. The vascular bundles of the peripheral ring are few or relatively numerous, uniformly small or varying in size; their sheaths, discrete or confluent. The structure of the stem epidermis is influenced by the absence of silica cells, the predominance of pointed cork cells, and other minor factors.

The node contains root band and growth ring. The latter may be wide or narrow, flush with the internode, constricted, or bulging. The root band may be high or narrow; of even height or taller on the bud side than on the opposite side; cylindrical, conoidal, or obconoidal. It contains one or several rows of root primordia, which are widely spaced or crowded, oval or round, dormant or with a tendency to sprout in the field.

The buds are placed alternately, each node bearing normally a single bud. They are inserted directly above the sheath scar, sometimes slightly lower or appreciably higher. The tip may or may not reach the growth ring but quite often projects some distance beyond. In some varieties, the buds are flush with the stalk or even depressed; in others, protruding. Color varies with variety and with age; often there is a contrasting coloration between side and wing tip in young buds. Buds may be large or small, short or long, flat or cushioned; their shape may be oval, ovate, obovate, pentagonal, rhomboid, triangular, or round.

The visible part of the bud is the prophyllum. It forms in its entirety an asymmetrical hood with the large front side composed of two overlapping halves and the short posterior side entire. The prophyllum consists of sides and wing; both show much variation in form and together determine the bud pattern. The edge of the overlapping half of the anterior side of the prophyllum forms a membranous margin that may terminate in an appendage. Other appendages may occur along the membranous margin, at times covering the germ pore or enlarging to form the beaked or rostellate bud forms. The wing of the bud may be inserted at, above, or below the center of the prophyllum; it may be wide or narrow, sometimes wider at the base than at the apex. The corners of the wing may be auriculate, the edges entire or emarginate. The tip of the bud is variously pointed, its margin smooth or notched. The venation of the prophyllum is

prominent or obscure, the veins straight or radially converging, the germ pore apical or central. The prophyllum may be practically smooth or pubescent, with the hairs arranged in more or less definite, distinct, or confluent groups; their occurrence and constancy are considered valuable taxonomic characters.

The leaves are two-ranked and consist of sheath and blade. At the juncture of sheath and blade is the blade joint with its specialized regions and at the base of the sheath is the leaf sheath node or sheath base.

The blade is erect, ascending, and gently curved, or erect with drooping tip. The surface is leathery, smooth, or rough; the veins conspicuous or faint. The midrib is either broad and massive or more or less shallow. The blade is relatively wide or narrow, sometimes reduced to the width of the midrib at the base. The edges are usually scabrous, the surface smooth except for microscopic hairs or a soft velvety pubescence. The upper and lower epidermis have each a characteristic pattern, varieties differing as to the presence of spines, stomatal grooves, and width of bulliform bands. The color of the blade is a shade of green, sometimes with a purplish cast.

The sheath forms an open tube, with the outer margin overlapping the inner. The outer margin is drawn out into a membranous border, which is often decurrent at the base and even appendaged. The leaves become abscised at the sheath base, leaving a characteristic scar that may be prominent or inconspicuous, straight or oblique, sometimes spreading and sagging under the bud. Sheath length is more or less constant within varietal limits. The surface is usually waxed and often covered with a vestiture of hair regionally distributed in definite groups. Characteristic hair groups are also found along the upper and lower decurrent sheath margin and at the base of the sheath joint. The epidermis shows broad intercostal regions and specialized zones overlying the veins. Varietal differences, based on the presence of spines and other characters, are valuable in classification.

The dewlaps, or joint triangles, are large or small, broad or very narrow, deltoid, squarish, or ligular. They are often asymmetrical. Their color differs considerably in young and mature organs and is subsequently altered by discolored wax deposits on the outer surface. Typical hair groups are present on both outer and inner surfaces. The ligule, a membranous appendage that separates the sheath from the blade, may be deltoid, strap-shaped, crescentiform, or arcuate, of uniform or asymmetrical construction; the free edge is often prominently ciliate and the back scabrous with free or partly adnate hairs. Back of the ligule, on the midrib, are a number of small hair groups constituting important taxonomic characters. Often there are present auricles, ear-shaped appendages of the sheath. In many varieties they are entirely wanting, in others they form transition structures; usually they are well developed, the inner almost always more prominently than the outer. There are many different morphological forms with variation in size within the different groups. The free edge of the auricle is usually ciliate, and the outer surface may, on rare occasions, bear tufts of long hair.

Characters differ qualitatively and in degree, and their stability is only relative. They represent the expression of inherited tendencies modified by environment. Characters that vary little are

usually associated with organs of ancient origin such as the ligule. The hair groups established by Jeswiet have a definite place in the classification scheme, but their value is only relative.

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STIGMA RECEPTIVITY AND POLLEN SHEDDING IN SOME PECAN VARIETIES ¹

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INTRODUCTION

The pecan (*Carya pecan* (Marsh.) Engl. and Graebn.) is a monoecious tree in which pollination is effected by the wind. The catkins, which bear the staminate flowers, arise from lateral, axillary, composite buds situated on the previous season's growth. These buds are formed as the shoot is produced, and the catkin primordia are differentiated at the time of bud formation when the subtending leaves are about one-tenth grown.² The catkin primordia occur on the sides of a central vegetative growing point within the composite group. The pistillate flowers are differentiated during the last of February and the first of March within terminal or lateral buds borne on shoots of the previous season.³ The pistillate flowers make their appearance soon after shoot elongation begins in the spring. Normal bearing trees may develop catkins from practically every node, but shoots that bear pistillate flowers are usually produced only from buds located at or near the terminals of the shoots.

The possibility that failure of self-pollination might result because of maturity and shedding of pollen either before or after the stigmas of the pistillate flowers are receptive was first investigated by Stuckey,⁴ who concluded that varieties of the pecan may be divided into two groups with respect to the time of pollen shedding and stigma receptivity. In one group, characterized by short, thick, compact catkins, he reported that pollen generally matured at about the time the stigmas became receptive; in the other group, characterized by relatively long, narrow, and less compact catkins, the stigmas became receptive 2 to 10 days before the staminate flowers shed their pollen. In this case a large percentage of the stigmas became dried or callused before the pollen was shed. However, Stuckey also stated that—

the difficulty in drawing definite conclusions * * * lies in the fact that in making field observations it is very difficult to determine just when the stigmas of pecans become receptive and the exact time when they pass the receptive stage.

Woodroof ⁵ gave dates of pollen shedding and of stigma receptivity for 4 years and indicated that some varieties are not capable of self-pollination in most seasons. He stated that the normal period of receptivity of a single stigma is about 5 days but that the length of the receptive period is very responsive to conditions of temperature and humidity. He concluded that pollen is shed immediately upon

¹ Received for publication October 9, 1939.

² WOODROOF, J. G. STUDIES OF THE STAMINATE INFLORESCENCE AND POLLEN OF HICORIA PECAN. Jour. Agr. Res., 40: 1059-1104 illus. 1930.

³ — and WOODROOF, NAOMI CHAPMAN. FRUIT-RUD DIFFERENTIATION AND SUBSEQUENT DEVELOPMENT OF THE FLOWERS IN THE HICORIA PECAN. Jour. Agr. Res. 33: 677-685, illus. 1926.

⁴ STUCKEY, H. P. THE TWO GROUPS OF VARIETIES OF THE HICORIA PECAN AND THEIR RELATION TO SELF-STERILITY. Ga. Expt. Sta. Bul., 124, pp. [127]-148, illus. 1916.

⁵ See footnote 2.

maturity of a catkin if conditions are suitable, but not if the temperature is too low or the atmospheric humidity above 85 percent.

Adrian⁶ gave the range of maturity of staminate and pistillate flowers for 6 years at College Station, Tex., and concluded that "the type of dichogamy in the pecan is not always fixed * * * there is a very strong tendency in certain seasons toward protandry and in others toward protogyny." On the basis of his data the varieties were classified into three groups: Protandrous, fluctuating, and protogynous. The shift in dichogamy was attributed to differences in response to varying weather conditions in late winter and early spring. He stated that "moisture and high temperature during the spring season favor early maturity of the staminate flowers, and cool, dry seasons favor the earlier maturity of the pistillate flowers."

Since definite knowledge of the dichogamy of varieties is necessary to insure adequate pollination in the pecan orchard, experiments were initiated to determine the periods of stigma receptivity by controlled pollination and the set of nuts. Previous workers determined the period of receptivity of pecan varieties by observation of the stigmas during their development. This method was not considered entirely reliable, because of the probability that pollination would alter the condition of the stigmas, and, as stated by Stuckey,⁷ it is extremely difficult to judge receptivity near the beginning or end of the period.

EXPERIMENTAL METHODS

In order to carry out the experiments under conditions of controlled pollination, it was necessary to cover the pistillate clusters to prevent wind-blown pollen from coming into contact with the stigmas. Traub and Romberg⁸ used small paraffined cloth bags as flower covers; but such bags were not entirely satisfactory, since they were relatively heavy, shaded the flowers, and held moisture to such an extent as to subject the pistillate clusters to an abnormally high humidity. It is not known how these conditions affect stigma receptivity. Therefore, in the work reported herein, bags made from viscose sausage casings were used. These were light in weight, transparent, and permitted diffusion of gases and water through them. In comparative tests these bags proved to be superior to the cloth bags in every respect. The size of bag found to be most satisfactory was $1\frac{1}{2}$ by $4\frac{1}{2}$ inches.

The bags were placed over the pistillate clusters before the stigmas were receptive and were tied securely upon a band of cotton placed around the tender young shoot for padding (fig. 1, A). Most of the small leaves near the base of the pistillate cluster were pinched off to facilitate covering. After these leaves are pinched off, the remaining leaves are stimulated into rapid growth and growth of other leaf and shoot buds is initiated. If the leaves or new shoots are allowed to develop to any appreciable extent in the bags, the pistillate clusters will abscise. Therefore, it is necessary to visit the bagged clusters once a week and pinch off the new leaves and shoot buds, which can be done without removing the bags.

⁶ ADRIAN, GUY W. FACTORS INFLUENCING FRUIT SETTING IN THE PECAN. *Bot. Gaz.* 91: 144-166, illus. 1931.

⁷ See footnote 4.

⁸ TRAUB, HAMILTON P., and ROMBERG, L. D. METHODS OF CONTROLLING POLLINATION IN THE PECAN. *Jour. Agr. Res.* 47: 287-296, illus. 1933.

Whenever possible, 4 trees and 100 clusters per tree were used for each variety. An experienced man was able to put on approximately 400 bags per day.

Pollinations were made by means of a hypodermic needle and syringe without removing the bags, the needle being inserted through the cotton pad. The syringe consisted of a rubber bulb, of the type used to water automobile batteries, and a one-hole stopper into which was inserted a glass tube (fig. 1, *B*). A 20-gage hypodermic needle $1\frac{1}{2}$ inches long was attached to the glass tube by means of rubber tubing. A loop in the glass tube was used to control the quantity of pollen applied per cluster. Numbered tags were attached to the shoots at the time of pollination to identify the clusters. The pollen was collected as follows. Mature catkins were picked and spread on

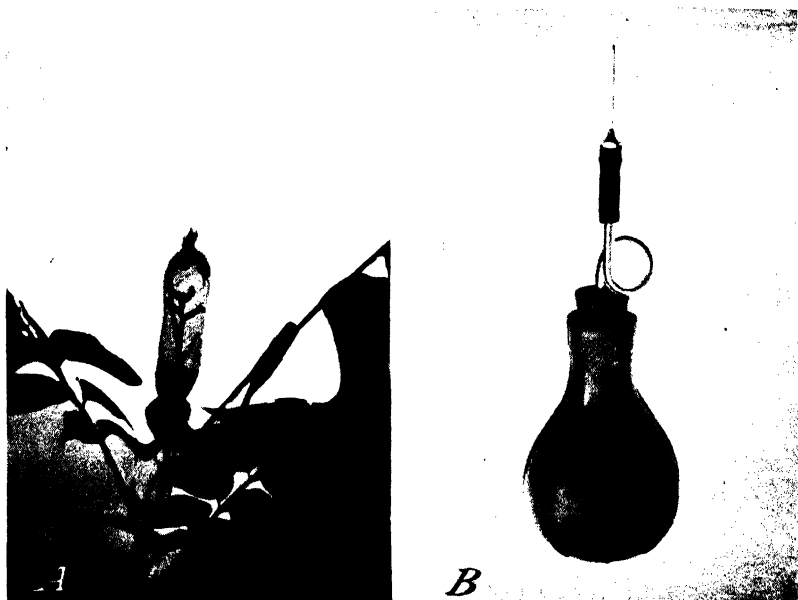


FIGURE 1.—*A*, Viscose bag for controlling pollination; inside the bag a leaf is growing, which if not pinched off might cause abscission of the pistillate cluster. *B*, Syringe used in pollinating bagged pecan flowers.

paper to dry. After 12 to 36 hours, the liberated pollen was collected and passed through cheesecloth or a fine-mesh sieve to remove foreign matter. The pollen was then placed in the rubber bulb of the syringe if it was to be used immediately. When it was to be used after 1 or more days it was spread in a thin layer in a covered cardboard box for storage. Thus kept, it was found to remain viable for not less than 7 days. Usually some old pollen was added to freshly sieved pollen in order to give greater bulk.

Pollination was commenced just before the beginning of stigma receptivity, as nearly as possible. On an average, about 16 clusters of each variety were pollinated each day during the period of receptivity. Random samples of nut clusters were obtained by pollinating them in the order of their occurrence in rotation about the trees.

Some clusters were left unpollinated to serve as checks on the degree of pollination control obtained and to indicate the time of dropping of unfertilized nuts.

As soon as the stigmatic surfaces had dried, indicating that receptivity had passed, the bags were removed and a record was taken of the number of nuts per cluster. A second count of the nuts in each cluster was made in late June or early July, which allowed sufficient time for the unfertilized nuts to drop. The nuts remaining at this time were taken as the set. From the data of the two counts the percentage of clusters setting nuts and the percentage of nuts set

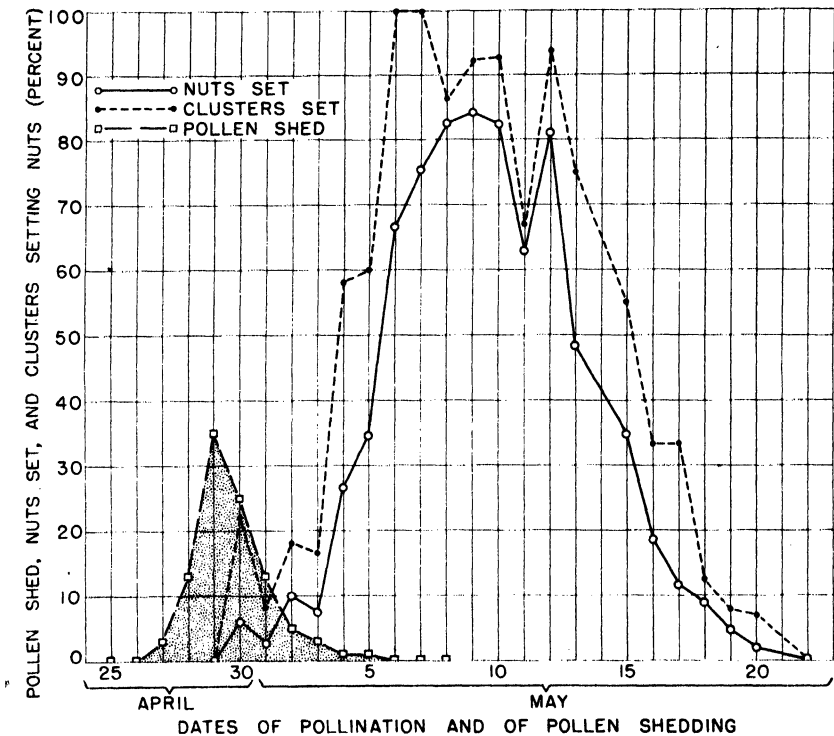


FIGURE 2.—Percentage of nuts set and of clusters setting nuts from controlled pollinations, and percentage of pollen shed on different dates during the season of 1933 by the San Saba Improved variety at Denison, Tex.

were calculated. In 1933 and in 1934, a larger number of clusters were left unpollinated and frequent counts of the nuts were made to determine more exactly the time that the unfertilized nuts dropped.

Owing to the relatively large size of the catkins, which ranged from 2½ to 6 inches in average length, it was easy to see the anthers, or portions of them, that had opened and liberated pollen. The amount of pollen that had been shed, expressed in percentage of the total on the tree, could be estimated. Such estimates were made daily for each tree. It is not presumed that these estimates are accurate; but this is the only simple quantitative measure that has been devised, and it is believed that it is a basis for better judgment of the polli-

nating possibilities of a variety than mere dates of first and last pollen shedding.

In 1933 the work was carried on in the Kemper orchard at Denison, Tex.; in 1934, at Austin; and in 1935, at Rogers. The Burkett, Sovereign (syn., Texas Prolific), and Schley varieties were used in each of the 3 years; Squirrel (syn., Squirrels Delight), Western (syn., Western Schley), San Saba Improved, Clark, Success, and Delmas, 2 years; and Stuart, Moneymaker, Kincaid, Jersey, Williamson, Halbert, and Onliwon, 1 year.

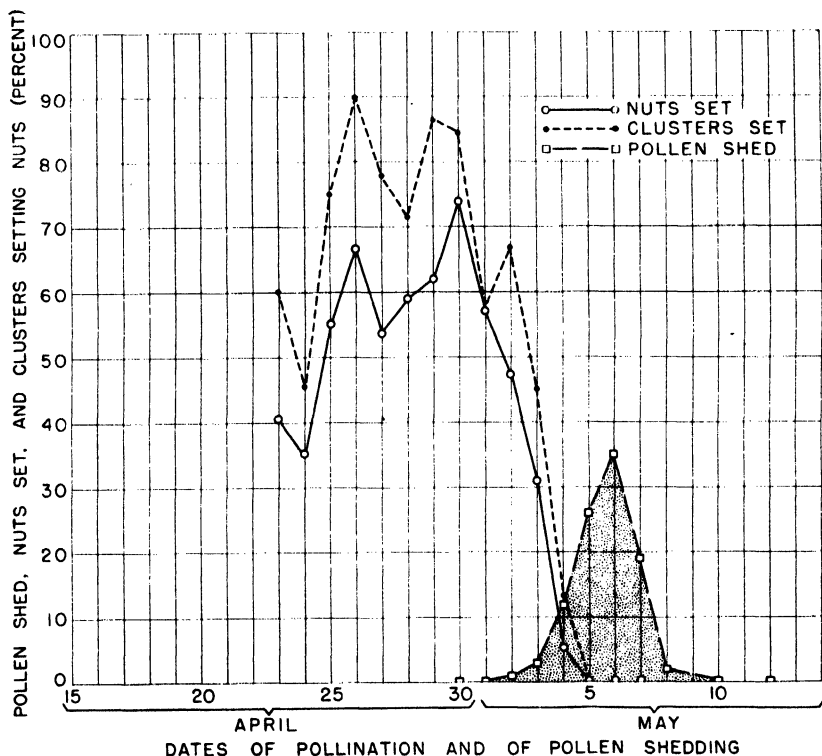


FIGURE 3.—Percentage of nuts set and of clusters setting nuts from controlled pollinations, and percentage of pollen shed on different dates during the season of 1934 by the Burkett variety at Austin, Tex.

In a study of dichogamy, made in 1935, the above-mentioned varieties were used and, in addition, the 11 following varieties: Humble, Bowers, Elgin, Russell, Odom, James, Teche, Alexander, Mosty, Mahan, and Curtis.

EXPERIMENTAL RESULTS

STIGMA RECEPTIVITY

Space does not permit the presentation of the detailed data obtained in the stigma-receptivity experiments during the three seasons. However, typical data for the percentage of nuts set and for the percentage of clusters setting nuts for each date of pollination are given in figures 2 and 3.

Clusters of San Saba Improved flowers were pollinated daily from April 29 to May 22 with the exception of May 14 and 21 (fig. 2). Beginning on May 1 and continuing for a period of 6 days, there was a rapid increase in the percentage of nuts set. This was followed by a period of 5 days during which a relatively high set of nuts was obtained, after which the percentage of nuts set decreased rapidly until May 17 and then more gradually to the end of the period. The total length of the stigma-receptivity period was 21 or 22 days. During the 7-day period from May 6 to 12, inclusive, the set of nuts might possibly have reached 100 percent had there been no losses of nuts from insect damage or causes other than lack of pollination.

The curve representing the percentage of clusters setting nuts shows the relationship between the clusters set and the nuts set. The percentage of clusters setting nuts was always higher than the corresponding percentage of nuts set. This was to be expected, since the setting of a single nut in a cluster gave 100-percent set on the cluster basis but usually much less on the nut basis, depending on the number of nuts in the cluster. In general, the data for other varieties were similar to those for the San Saba Improved except for the lengths of the receptive periods of the stigmas.

The pistillate flowers were not always pollinated early enough to include the first dates of stigma receptivity, and in a few cases pollinations were not made late enough to include the last dates of stigma receptivity. However, this is not considered of much importance, since near the beginning and end of the period only a few stigmas are receptive. The important dates within the range of receptivity of all flowers of a variety are those when a relatively high percentage of the flowers are receptive; therefore the approximate time during which 50 percent or more of the stigmas were receptive, as indicated by the set of nuts, was arbitrarily selected as the important period within the range of receptivity and will be referred to as the major receptive period.

The major receptive periods were determined by the set of nuts obtained in the day-to-day pollinations. Since it was apparent that causes other than the lack of fertilization prevented a set of 100 percent of the flowers pollinated during the period of high set, except in rare cases, 50 percent of the stigmas of a variety were considered receptive when the percentage set of nuts was at least one-half of the average set during the period of high set. For example, the San Saba Improved variety (fig. 2) set an average of 78 percent of the nuts pollinated from May 7 to 12, the period of high set. Thus the period from the 39-percent point on the upward slope to the 39-percent point on the downward slope of the receptivity curve represents the major receptive period of the stigmas, and extends in this case from May 5 to about May 14, or 9 days. This method could not be followed exactly in all cases, owing to fluctuations or incomplete data, and it was then necessary to make estimates. The major receptive periods, together with the first and last determined dates of stigma receptivity for the varieties used in this experiment, are given in table 1, and the major receptive periods are shown graphically in figure 4.

In most cases the length of the major receptive period varied from season to season in the same variety and in different varieties in the same season. The shortest period was 8 days for Stuart and Success

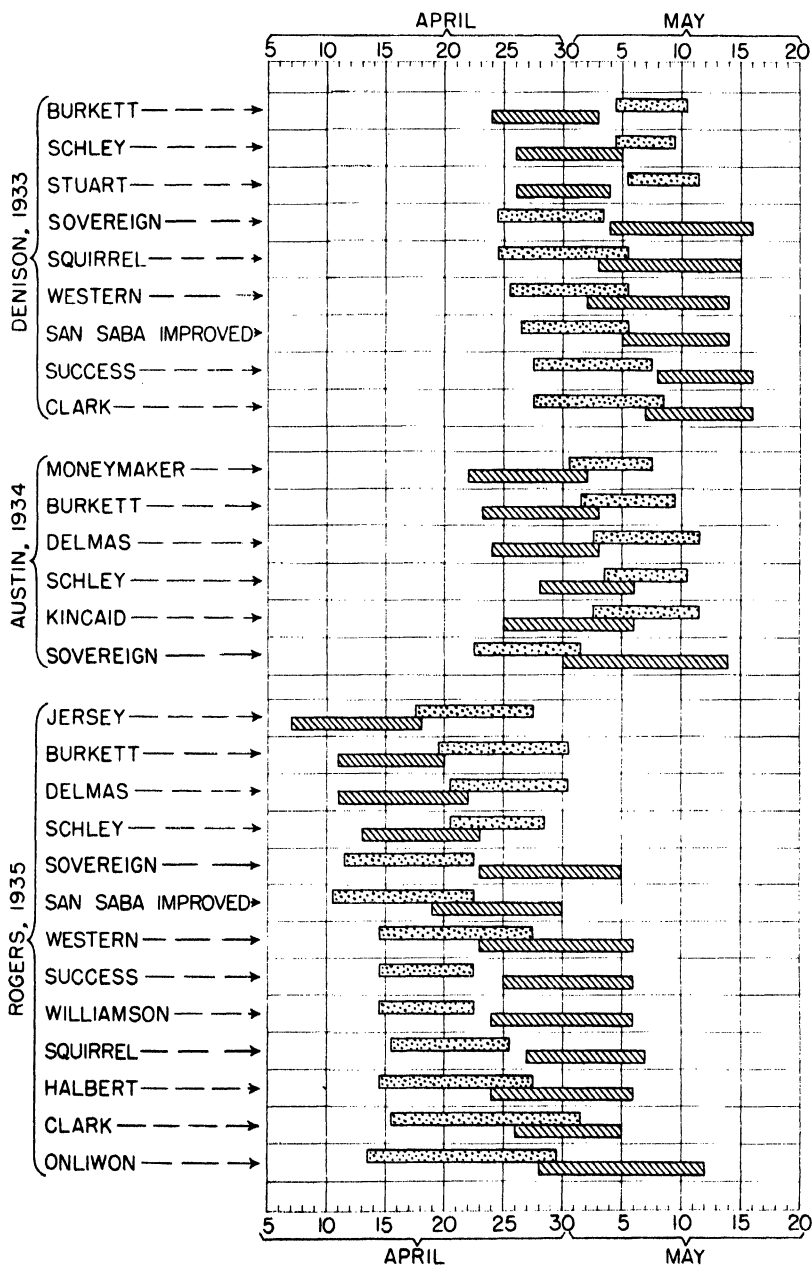


FIGURE 4.—Major periods of stigma receptivity and of pollen shedding in some varieties of the pecan, Texas, 1933-35. Dotted rectangles represent pollen shedding; cross-hatched rectangles represent stigma receptivity.

in 1933 and for Schley in 1934; the longest period was 14 days for Sovereign in 1934 and for Onliwon in 1935.

Studies made on 12 varieties showed that a period of 5½ to 6½ weeks after the date of last receptivity was required for all unfertilized nuts to drop.

TABLE 1.—*Time and duration of stigma receptivity of pecan flowers of several varieties during 3 seasons*

DENISON, 1933

Variety	Date of first stigma receptivity	Date of last stigma receptivity	Dates of major period of stigma receptivity ¹	Length of major period of stigma receptivity Days
Burkett		May 4	Apr. 24-May 3	9
Schley	Apr. 24	May 7	Apr. 26-May 5	9
Stuart			Apr. 26-May 4	8
Sovereign	Apr. 30	May 17	May 4-16	12
Squirrel		do	May 3-15	12
Western	Apr. 30	do	May 2-14	12
San Saba Improved	do	May 20	May 5-14	9
Success	do	May 18	May 8-16	8
Clark	May 4	May 22	May 7-16	9

AUSTIN, 1934

Moneymaker		May 3	Apr. 22-May 2	10
Burkett		May 4	Apr. 23-May 3	10
Delmas		May 8	Apr. 24-May 3	9
Schley		May 7	Apr. 28-May 6	8
Kincaid			Apr. 25-May 6	11
Sovereign		May 17	Apr. 30-May 14	14

ROGERS, 1935

Jersey		Apr. 22	Apr. 7-18	11
Burkett		do	Apr. 11-20	9
Delmas		Apr. 27	Apr. 11-22	11
Schley		Apr. 26	Apr. 13-23	10
Sovereign	Apr. 22	May 8	Apr. 23-May 5	12
San Saba Improved		May 6	Apr. 19-30	11
Western	Apr. 19	May 12	Apr. 23-May 6	13
Success	Apr. 21	May 9	Apr. 25-May 6	11
Williamson	Apr. 23	May 10	Apr. 24-May 6	12
Squirrel	Apr. 24	May 11	Apr. 27-May 7	10
Halbert		May 10	Apr. 24-May 6	12
Clark	Apr. 24	do	Apr. 26-May 5	9
Onliwon	Apr. 26	May 16	Apr. 28-May 12	14

¹ The major period of stigma receptivity is the period during which the daily set of nuts was 50 percent or more of the average daily set during the period of high set. This period extends from the first to the last date given and is not inclusive of both dates.

POLLEN SHEDDING

Pollen shedding normally begins gradually and ends somewhat more gradually, whereas in the middle of the period it is usually very rapid (figs. 2 and 3). In general, the earliest pollen is shed by catkins on a few branches somewhat low down and toward the inside of the tree, which are especially early in commencing growth in the spring (fig. 5). The last catkins to shed pollen are those on water sprouts growing on the trunk or large limbs of the tree or those on vigorous long shoots of the previous season, which start growth late and generally

are found on the outside and toward the top of the tree. The extent to which catkin development on a tree may vary is shown in figure 6.



FIGURE 5.—Young pecan tree at the beginning of growth in the spring, showing the extremes of variation in shoot development.

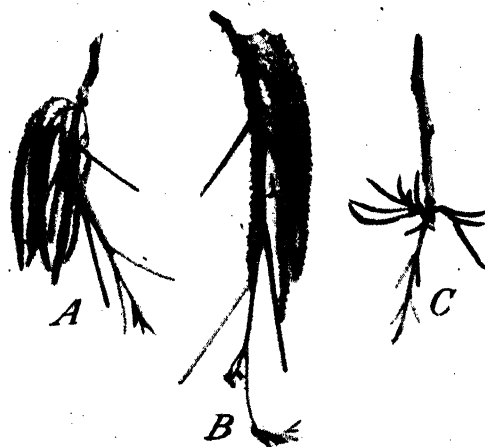


FIGURE 6.—Branches selected from a pecan tree on the same date, showing the extremes sometimes found in development of catkins and pistillate clusters and also showing the correlation in development of staminate and pistillate flowers originating from the same compound bud: A, Average; B, early; C, late.

It requires close observation to determine when pollen is first liberated and when shedding is complete. Therefore, the dates given as the first and last dates of shedding depend considerably upon the observer. It is often difficult to set any particular day as the last, owing to the presence of weak, poorly developed catkins, some of which may mature pollen still later. Also, a few pollen grains that have been retained or lodged in the catkins continue to be released. There is a strong tendency to disregard these small quantities.

In these experiments an effort was made to observe the very first and the very last pollen to be shed. Estimates also were made of the time interval from the date at which 1 percent of all pollen had been shed to the date at which 99 percent had been shed by each variety; this period, inclusive of both dates, will be referred to as the major pollen-shedding period. Under average orchard conditions it is doubtful if the first 1 percent or the last 1 percent of pollen is shed daily in sufficient quantities to effect a good set of nuts, whereas, in the interval between the time when 1 percent and 99 percent has been shed, the quantity shed daily should be sufficient to pollinate all receptive stigmas within range of the trees of the variety shedding pollen.

The estimated dates of first and last pollen shedding and of the major pollen-shedding periods are given in table 2. There were wide

TABLE 2.—*Dates and duration of the periods of pollen shedding of several varieties of pecan during 3 seasons*

Variety	Dates of first and last observed pollen shedding	Major pollen-shedding period ¹		Total length of pollen-shedding period
		Dates	Length of period	
			Days	Days
Burkett.....	May 4-11.....	May 5-10.....	6	8
Schley.....	May 3-10.....	May 5-9.....	5	8
Stuart.....	May 5-12.....	May 6-11.....	6	8
Sovereign.....	Apr. 24-May 7.....	Apr. 25-May 3.....	9	14
Squirrel.....	Apr. 24-May 8.....	Apr. 25-May 5.....	11	15
Western.....	Apr. 25-May 8.....	Apr. 26-May 5.....	9	14
San Saba Improved.....	do.....	Apr. 27-May 5.....	9	14
Success.....	Apr. 26-May 10.....	Apr. 28-May 7.....	10	15
Clark.....	do.....	Apr. 28-May 8.....	11	15
DENISON, 1933				
AUSTIN, 1934				
Moneymaker.....	Apr. 29-May 12.....	May 1-7.....	7	14
Burkett.....	Apr. 30-May 12.....	May 2-9.....	8	13
Delmas.....	May 1-14.....	May 3-11.....	9	14
Schley.....	May 1-12.....	May 4-10.....	7	12
Kincaid.....	Apr. 30-May 12.....	May 3-11.....	9	13
Sovereign.....	Apr. 21-May 3.....	Apr. 23-May 1.....	9	13
ROGERS, 1935				
Jersey.....	Apr. 17-May 2.....	Apr. 18-27.....	10	16
Burkett.....	Apr. 18-May 5.....	Apr. 20-30.....	11	18
Delmas.....	Apr. 18-May 1.....	Apr. 21-30.....	10	14
Schley.....	Apr. 18-30.....	Apr. 21-28.....	8	12
Sovereign.....	Apr. 10-25.....	Apr. 12-22.....	11	16
San Saba Improved.....	Apr. 10-23.....	Apr. 11-22.....	12	14
Western.....	Apr. 13-May 2.....	Apr. 15-27.....	13	20
Success.....	Apr. 14-26.....	Apr. 15-22.....	8	12
Williamson.....	Apr. 14-24.....	do.....	8	11
Squirrel.....	Apr. 16-30.....	Apr. 16-25.....	10	15
Halbert.....	Apr. 16-May 2.....	Apr. 15-27.....	13	21
Clark.....	Apr. 12-May 7.....	Apr. 16-May 1.....	16	26
Onliwon.....	Apr. 11-May 2.....	Apr. 14-29.....	16	22

¹ Estimated dates and duration of period from the time 1 percent to the time 99 percent of pollen was shed. The major period includes first and last dates as given in column 3, since each date represents the preceding 24-hour period during which at least 1 percent of the pollen was shed.

differences in the lengths of the pollen-shedding periods of the different varieties in the same season as well as wide differences within the same variety in different seasons. For instance, the Burkett variety shed pollen over a period of 8 days in 1933, 13 days in 1934, and 18 days in 1935, while the major pollen-shedding periods for the same years were 6, 8, and 11 days, respectively. The Clark variety shed pollen over periods of 15 and 26 days, respectively, in 1933 and 1935, while the corresponding major periods were 11 and 16 days. The total length of the pollen-shedding periods varied from 8 days for the Burkett, Schley, and Stuart varieties in 1933 to 26 days for the Clark variety in 1935, while the major periods varied from 5 days for the Schley variety in 1933 to 16 days for the Clark variety in 1935. The three series of experiments were conducted in different years, in different localities, and with trees having different types of shoot growth. These factors probably were largely responsible for the variations in pollen-shedding periods of the same variety in the different years.

DICHOGAMY

PROTANDROUS AND PROTOGYNOUS VARIETIES

The relationship of the major pollen-shedding period to the major receptive period of each variety is shown graphically in figure 4. In no instance are the two periods closely concurrent, and in several cases they do not overlap at all. The varieties fall into two groups with reference to the relative time at which pollen is shed as compared with the time the stigmas are receptive. In one group (protandrous) the pollen is shed early and in the other group (protogynous), it is shed late, as compared with stigma receptivity.

The staminate flowers of the protandrous varieties develop more rapidly than those of the protogynous varieties, but the pistillate flowers develop in the reverse order. As a general rule, pollen is shed earlier and stigma receptivity is later in protandrous varieties than in protogynous varieties (fig. 4). However, the time at which a variety matures its staminate and pistillate flowers in any season depends to a large extent upon the time of growth initiation in the spring. In extreme cases the pollen-shedding period of a protandrous variety may coincide with that of a protogynous variety if the protandrous variety starts growth late in the spring. Likewise the pollen-shedding period of an especially early protogynous variety may coincide with that of a protandrous variety or may be even earlier than some protandrous varieties.

DEGREE OF DICHOGAMY IN DIFFERENT VARIETIES

During the course of these experiments it became apparent that the degree of dichogamy was not the same in all varieties. Experiments to determine this point more definitely were carried out in 1935. In the case of protogynous varieties, pistillate clusters were bagged before the stigmas were receptive. Later some of these clusters were pollinated when observations indicated that a high percentage of the stigmas were receptive; the others were not pollinated until just at the time pollen was being shed by the catkins on the same twigs that bore the pistillate clusters. It was assumed that varieties setting a relatively high percentage of the nuts pollinated at that time could be considered

self-fruitful during that year. The data obtained (table 3) show that in only one variety was the set of nuts as high from pollinations made at the time of pollen shedding as from earlier pollinations. In several of the varieties dichogamy was complete, no nuts being set when pollinations were delayed until the pollen had begun to shed; in other varieties the degree of dichogamy varied greatly. In the Russell, Odom, Kincaid, Delmas, and Schley varieties the degree of dichogamy was less than in the others used.

TABLE 3.—*Relative degree of dichogamy in several pecan varieties in the protogynous group, season of 1935*

Variety	Pollinated before pollen shed by catkins on same branch ¹				Pollinated at start of pollen shedding on same branch ¹			
	Period of pollina- tion	Nuts used	Nuts set		Period of pollina- tion	Nuts used	Nuts set	
		Number	Number	Percent		Number	Number	Percent
Humble	Apr. 12	83	24	28.9	Apr. 20-24	92	0	0.0
Bowers	do	59	2	3.4	Apr. 21-25	66	0	0.0
Elgin	do	39	12	30.8	Apr. 22-25	66	0	0.0
Russell	Apr. 18	17	7	41.2	Apr. 22-24	23	9	39.1
Odom	Apr. 16-20	31	14	45.2	do	23	5	21.7
Kincaid	Apr. 18	63	16	25.4	Apr. 23-27	93	25	26.9
James	do	85	33	38.8	Apr. 24-27	121	1	.8
Teche	do	76	20	26.3	do	78	0	0.0
Alexander	Apr. 20	37	24	64.9	do	44	8	18.2
Mosty	Apr. 18	19	17	89.5	Apr. 23-26	74	12	16.2
Mahan	Apr. 16-20	146	60	41.1	Apr. 24-28	112	5	4.5
Stuart	Apr. 25	58	22	37.9	Apr. 24-May 1	83	4	4.8
Curtis	Apr. 27	44	26	59.1	Apr. 29-May 6	44	2	4.5
Jersey	Apr. 10-12	184	81	44.0	Apr. 17-23	48	0	0.0
Burkett	Apr. 14-16	74	27	36.5	Apr. 18-25	60	0	0.0
Delmas	Apr. 16-18	125	37	29.6	Apr. 19-24	37	8	21.6
Schley	do	96	28	29.2	Apr. 22-24	39	7	17.9

¹ The branch referred to is the twig of the previous season's growth from which grew the current shoots bearing pistillate clusters.

In the protandrous varieties, shoots were tagged at the time pollen shedding began and the time interval between this date and that when the first stigmas on these branches were receptive was determined. Stigma receptivity was found by pollinating and then bagging different pistillate clusters at varying intervals after the beginning of pollen shedding, and later counting the number of nuts set. The data obtained were insufficient to warrant conclusions, but, together with the data secured in the receptivity experiments, they indicate that the degree of dichogamy in this group varies as much as in the protogynous varieties. The degree of dichogamy of a variety may vary from season to season, but in no case was it found to vary enough to shift the type of dichogamy from protandrous to protogynous, or vice versa.

DISCUSSION

The graphs representing the set of nuts from day-to-day pollinations show considerable fluctuation (figs. 2 and 3). These fluctuations are ascribed chiefly to nonuniformity in the samples, to loss of nuts from insect damage, and to other conditions, not associated with the lack of pollination or fertilization, that prevailed generally after the bags were removed and before the set of nuts could be recorded. In general, the receptivity curves of all varieties were of the same form, showing a relatively high set of nuts over a considerable time within the recep-

tive period and indicating a relatively long period of receptivity of individual stigmas. Since the percentage of clusters set varied approximately with the percentage of nuts set, it is also indicated that all stigmas in a cluster became receptive within a very short time. Further experiments are being conducted to determine more definitely the length of the receptive periods of individual stigmas and the variation in time at which the individual stigmas in a cluster become receptive.

There was considerable variation in the total length of the period of stigma receptivity of a given variety in different years and also a difference in the total length of the periods of stigma receptivity of different trees in the same year. The differences occurring in the same year are attributed largely to variations in growth and development of the shoots bearing the pistillate clusters, and the differences occurring in different years may be influenced by these factors and by seasonal variations. The extent to which the development of the shoots sometimes varied from the average is shown in figure 6. It was observed also that buds starting growth early were early in maturing flowers, and vice versa. Therefore, the lack of uniformity in the development of the stigmas probably was due largely to the difference in time at which the buds producing pistillate shoots started growth. The interval between the time of initiation of growth of the earliest and latest buds on a tree varied somewhat according to the way the tree had grown the previous season. The shoots on slow-growing trees were more uniform in initiation of growth and flower development than those on fast-growing trees. Therefore, the total length of the receptive period of the stigmas varied with different trees of a variety, and when there was a difference of several days in the time of general growth initiation in the trees the receptive period was longer. This variation might also affect the length of major periods of stigma receptivity, but these were usually affected less than the total receptive periods. The same relationship held in the development of the staminate flowers and the shedding of pollen.

It was observed that a period of rain or fog halted the opening of the anthers, but apparently the high humidity affected only the mechanism of anther dehiscence and not the pollen-ripening processes, since such periods were generally followed by unusually heavy pollen shedding. During the 3 years of the experiment there were never 2 consecutive days during which no pollen was shed on account of rain or high humidity.

The degree of dichogamy varied widely but was high in most of the varieties used in these experiments. Therefore, in planting a pecan orchard or in top-working a seedling grove provision should be made to insure adequate pollination by the use of two or more varieties selected so that pollen will be shed during the major receptive periods of all the varieties used. In general, a good crop of nuts is possible if 50 percent or more of the stigmas on the trees set nuts, and at least 50 percent of the stigmas should be pollinated if the major periods of receptivity and pollen shedding overlap by 1 or more days when conditions are favorable for pollen shedding. However, it is desirable that pollen be shed throughout the major periods of receptivity of all varieties, and this condition is attained by selection and interplanting of the proper protogynous and protandrous varieties. It is noted

from the data (fig. 4) that in general the protogynous varieties shed pollen at a time when a high percentage of the stigmas of the protandrous varieties were receptive, and vice versa. However, this may not always occur, because it is possible for the pollen-shedding periods of some protogynous varieties to coincide with those of some protandrous varieties, as previously pointed out. In such a case neither variety would furnish adequate pollen for the other, because the receptive period of the stigmas of the protogynous variety would be almost past before pollen shedding began and receptivity of the stigmas of the protandrous variety would be just beginning at the time pollen shedding was completed.

If climatic variations in the spring cause a change in the interval between the staminate and pistillate flowering periods that is favorable to protogyny or protandry, it is assumed that all varieties will be shifted in the same direction, though possibly not to the same degree. If this is true, the interval between the pistillate and staminate flowering periods should be increased in protandrous varieties in a season favoring protandry but decreased in protogynous varieties in such a season. Similarly the interval should be decreased in protandrous varieties in a season favoring protogyny but increased in protogynous varieties under the same conditions. A study of the data, which are not entirely consistent, indicates neither a pronounced nor a uniform shift toward either protandry or protogyny in any variety during the 3 years.

SUMMARY

The receptive periods of the stigmas of 16 varieties of the pecan were determined during one to three seasons. There was a wide variation in the length of the periods of stigma receptivity of different varieties in the same year and of the same variety in different years. The variations in the same variety may be ascribed to variation in time of initiation of growth of buds producing pistillate shoots and to seasonal variations.

Studies made on 12 varieties showed that all unpollinated nuts dropped within a period of 5½ to 6½ weeks after the last date of stigma receptivity.

The range in maturity of staminate flowers varied in the same way as the pistillate flowers, but pollen shedding was irregular owing to changes in weather conditions.

Pecan varieties may be placed in two groups according to blossoming characteristics. In one group, pollen shedding occurs relatively early and stigma receptivity relatively late; in the other, the relative time of maturity of the flowers is reversed. However, the actual time at which the flowers of one tree mature as compared with those of other trees is also dependent on the time of growth initiation and the relative rate of shoot growth and flower development.

The degree of dichogamy of different varieties was found to vary widely. In some instances sufficient coordination existed for a good set of nuts by self-pollination, whereas in other cases only a very little or no self-pollination was evident.

No case of a pronounced shift in dichogamy, either toward protandry or protogyny, was found in any variety during the three seasons of the experiment.

INEFFECTIVENESS OF PROPRIETARY REMEDIES AND OTHER DRUGS IN THE CONTROL OF BANG'S DISEASE, WITH SPECIAL REFERENCE TO "3-V TONIC" AND "BOWMAN'S"¹

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USE OF THERAPEUTIC AGENTS IN BANG'S DISEASE

In the investigation of Bang's disease by Federal and State experiment stations and some privately operated laboratories, much effort has been spent in studying the effect of various drugs on this disease. It was hoped to find some agent which could penetrate the tissues of the affected animal in sufficient concentration to destroy the *Brucella abortus* germs causing the disease without damage to the tissues themselves. A large number of different chemical substances which have recognized therapeutic value have been tried by various experiment stations and laboratories, but not one of these products has been proved, in controlled experiments, to have any value in the prevention or cure of Bang's disease. Recently, sulfanilamide, a chemical product which has been found to be very effective in the treatment of certain streptococcic infections and which has been reported to be of some benefit in the treatment of brucellosis in man, was tried in cattle affected with Bang's disease. Although the quantity fed was only slightly less than the toxic dosage, it had no apparent action in destroying the *Brucella* organisms or reducing their number or virulence.

Thus the history of drugs or other therapeutic chemicals in the treatment of Bang's disease has been negative so far as finding any substance which has a specific action on *Brucella* organisms in the tissues of animals. These results have been published from time to time in veterinary literature, but in spite of this fact so-called remedies for Bang's disease still appear on the market. Many thousands of dollars have been spent by farmers for such worthless products.

CONTROL OF CONCERNS PRODUCING BANG'S DISEASE REMEDIES

Remedies for Bang's disease, commonly called abortion cures, are subject to control under the Federal Food and Drug Act. Although the United States Food and Drug Administration has been active in the enforcement of that law as it relates to such products, the statute does not cover advertising as such or products marketed within the State in which they are produced. Protection against false advertising of such products is afforded, however, under the Wheeler-Lea Amendments enforced by the Federal Trade Commission.

Undoubtedly the most important factor in the sales promotion of Bang's disease remedies is literature containing testimonials from

¹ Received for publication April 26, 1939.

livestock owners who have used such products in their infected herds. The sincerity of the writers of these testimonials is not questioned by investigators who have studied the nature of Bang's disease, but these investigators know that any apparent improvement in an affected herd following the use of the "cure" was not due to medication, but was a natural sequence in the course of the disease.

NATURE OF BANG'S DISEASE

When Bang's disease first appears in a herd, it is followed by a wave of abortions in the pregnant animals. This period lasts for 1 or 2 years, or until all susceptible pregnant animals have contracted the disease. This, known as the active stage of the disease, is followed by the chronic stage. When cows have contracted the disease and aborted, many will still harbor the *Brucella* organisms, but in spite of this fact—and this point is to be especially noted—they rarely abort at subsequent pregnancies. This ability of the animals to carry full-term calves in spite of being infected is due to immunological changes which have developed within their bodies. During the active stage of Bang's disease, 15 to 50 percent of the pregnant animals will abort. In some herds the percentage is much higher. When the disease has reached the chronic stage, however, there may be no abortions or only a small percentage, most of which are confined to replacements in the herd or heifers subjected to exposure for the first time. An owner does not use a "cure" if his herd is free from the disease. Generally before it is used, the disease will be approaching the end of the active stage. Thus after using the cure, the owner will note that the abortions have greatly decreased or practically ceased, and it is only natural that he should assume that the remedy was responsible for the apparent improvement of the herd. However, his herd is still infected and will remain so until the animals carrying the infection are removed.

PURPOSE OF TESTS OF SO-CALLED CURES

Federal and State investigators of Bang's disease and State livestock sanitary officials have long known that hundreds of farmers and herd owners have been and still are paying out their hard-earned money for the purchase of so-called remedies which are worthless in the prevention or cure of this disease. Authorities are powerless, however, to stop such sales in the absence of direct violation of Federal or State law. To prevent further losses to owners of infected herds through the purchase of such products, the Congress of the United States passed an amendment to the Agricultural Administration Act permitting a portion of the funds appropriated under this act to be spent in testing various proprietary remedies alleged to be preventives or cures for Bang's disease.

Obviously, it was impracticable to submit all alleged remedies for Bang's disease to controlled tests with the money made available. Therefore, two products which were believed to be the most widely advertised and used were selected for test. These products were (1) 3-V Tonic, manufactured by the Crawford Co., Winona, Minn., and (2) Bowman's, manufactured by the Bowman Laboratory, Owatonna, Minn.

These tests ² were made in the State of Wisconsin in 1936 and 1937. Professional employees of both the Bureau of Animal Industry and the University of Wisconsin participated in their planning and performance.

A description of the testing of each of these products, covering the history and treatment of each animal, a chemical analysis of the respective products, and a summary of results, is given in the following pages.

CRAWFORD 3-V TONIC

The 3-V Tonic was obtained from T. E. Crawford, of the Crawford Co. An analysis of the product made by the United States Food and Drug Administration showed it to consist essentially of lime, basic magnesium carbonate, charcoal, a soluble compound of sulfur and calcium, probably calcium hyposulfite, small proportions of ferrous sulfate, sulfur, and powdered linseed, and a smaller proportion of powdered ginger.

It was claimed for the 3-V Tonic that it would prevent abortion and enable cattle affected with Bang's disease to bear normal, healthy, full-term calves. To determine whether the claims made were based on facts, and also whether the product would prevent or cure Bang's disease, the following experiment was made.

EXPERIMENTAL PROCEDURE

Sixty heifers that were believed to be pregnant were purchased from herds with a negative history of abortions. These herds had previously been subjected to from one to five Bang's disease tests, and no evidence of infection had been found. The heifers gave negative results to the Bang's disease test at the time they were obtained. They were placed on a farm that had been rented for the experimental work. It was located a few miles from the University of Wisconsin. The heifers were examined for pregnancy before they were purchased. They were divided into three groups similar as to condition but were kept in the same barn and allowed to mingle in an exercising yard.

To determine its preventive effects, the 3-V Tonic was fed to 20 heifers in group 1 before they were exposed to *Brucella abortus*. To determine the curative effects, group 2, likewise consisting of 20 heifers, was fed the product after exposure to *Br. abortus*. Group 3 received none of the product but served as controls on the other two groups.

Beginning with the evening feeding on October 12, 1936, the product, mixed with the morning and evening feed, was fed regularly to group 1 in accordance with directions prescribed by the manufacturer. Each animal received one-half ounce (1 tablespoonful) of the material daily for 75 days. The total quantity fed to each animal during the entire period was 2½ pounds.

² The tests were planned and directed by W. E. Cotton, superintendent, and J. M. Buck, assistant superintendent, of the Animal Disease Station, Bureau of Animal Industry, with the cooperation of E. G. Hastings, B. A. Beach, and Noble Clark, of the Wisconsin Agricultural Experiment Station. Following the retirement of Dr. Cotton, September 30, 1937, Dr. Buck assembled the data for this report. After the death of Dr. Buck on May 2, 1938, A. B. Crawford, of the Animal Disease Station, completed the report, which was reviewed and approved, with slight revision, by the representatives of the Wisconsin Agricultural Experiment Station. (There is no relation between A. B. Crawford, the senior author of this paper, and T. E. Crawford, of the Crawford Co., the producer of 3-V Tonic, the similarity of names being coincidental.) J. S. Healy, of the U. S. Bureau of Animal Industry, Madison, Wis., supervised the feeding and management of the experimental herds.

Feeding of the product to group 2 was begun with the evening feeding of November 28, 1936, the day the animals were subjected to *Br. abortus* exposure, and continued for 75 days, the animals in this group receiving the same doses and in the same manner as those in group 1.

On November 28, 1936, when the heifers in group 1 had been fed the product for 48 days, all the animals in the three groups, with the consent of T. E. Crawford, were exposed to *Brucella abortus*. The feeding of the product to the animals in group 1 was then continued for 27 days in order to complete a 75-day feeding period.

The exposure of the cattle to *Brucella abortus* was made by representatives of the United States Bureau of Animal Industry and the University of Wisconsin in the presence of T. E. Crawford, who, after it had been completed, expressed himself as considering it very fairly carried out.

The method of exposing the heifers to Bang's disease was through the mucus membrane of the eye. A suspension of *Brucella abortus* (bovine) was prepared with three strains of the micro-organism, designated, respectively, 213, 1927, and 214. Strain 213 had been isolated, about 1 year previously, from the lymph glands associated with the udder of an infected cow; strain 1927 had been recently isolated from an aborted fetus; and strain 214 had been recently isolated from the milk of an infected cow. Three small drops of the suspension were instilled in the mucous membrane of one eye of each animal. In no instance was any of the suspension seen to escape from the eye cavity. Use was made of but 8 cc. of the suspension to expose the 60 heifers, each heifer receiving 0.13 cc. of the material. This method of exposure to Bang's disease has been extensively used in vaccination experiments conducted by the United States Bureau of Animal Industry because it provides a dependable way of transmitting the disease to pregnant cattle that possess normal resistance to it.

All the heifers gave negative results to the agglutination test for Bang's disease on December 4, 1936, 6 days after they were exposed. On the next test, which was made 13 days after they received eye exposure, all 60 animals reacted to the test in titers ranging from 1 to 50 to 1 to 400. Their reactions, in nearly all cases, gradually increased in intensity until a titer of 1 to 6,400 or higher was reached.

When the gestation period of each heifer terminated, several guinea pigs were injected with a sample of her uterine material and others with a composite sample of her colostrum to determine whether *Brucella abortus* was present in these substances. In the event that the heifer aborted, the fetus was cultured.

RESULTS OF THE EXPERIMENT

One of the heifers in group 1, treated with the product before exposure, had to be eliminated from the experiment because of accident and one control proved to be nonpregnant. There were, therefore, 58 heifers which completed the experiment and for which results are available. These are shown in table 1. Fifty-two of the animals aborted, and 6 gave birth to weak calves with insufficient vigor to survive. *Brucella abortus* was isolated from all but 6 of the fetuses—3 of the controls and 3 of the group that was fed the product after

TABLE 1.—*Results of feeding Crawford 3-V Tonic to pregnant heifers to prevent or cure Bang's disease*

[Date of *Brucella abortus* eye exposure in the 3 groups, Nov. 28, 1936; in group 1, date of first feeding, Oct. 12, and date of last feeding, Dec. 25, 1936; in group 2, date of first feeding, Nov. 28, 1936, and date of last feeding, Feb. 11, 1937]

GROUP 1.—FED PRODUCT 48 DAYS PRIOR TO AND 27 DAYS SUBSEQUENT TO EXPOSURE

Animal designation	Date of termination of gestation	Outcome of pregnancy	Approximate stage of pregnancy	Results of examination for <i>Brucella abortus</i> ¹	
				Uterus	Colostrum
DJ 14304	1936 Dec. 27	Abortion	Months 6	+	+
DK 64423	1937 Jan. 2	do	7 $\frac{1}{2}$	+	+
DH 74369	Jan. 4	do	6 $\frac{1}{2}$	+	+
DH 65809	Jan. 5	do	8 $\frac{1}{2}$	+	+
DE 54524	Jan. 11	do	6	+	+
DK 74136	Jan. 13	do	6	+	o
DS 87971	Jan. 14	do	5 $\frac{1}{2}$	+	+
DA 75155	do	do	7 $\frac{1}{2}$	+	o
DH 62400	do	do	7	+	+
DH 80354	Jan. 15	do	7 $\frac{1}{2}$	+	+
DH 80363	Jan. 18	do	8	+	o
DE 15741	do	do	7	+	+
DJ 65870	Jan. 20	do	6 $\frac{1}{2}$	+	o
DJ 14305	Jan. 26	do	7	+	+
DS 87969	Jan. 27	do	6	+	+
DK 64363	Feb. 1	do	6 $\frac{1}{2}$	+	+
DK 64888	Feb. 4	do	6 $\frac{1}{2}$	+	o
DH 46564	do	do	7	+	o
DS 33355	Feb. 7	do	6 $\frac{1}{2}$	+	+

GROUP 2.—FED PRODUCT SUBSEQUENT TO EXPOSURE ONLY

DS 45047	1936 Dec. 27	Abortion	6 $\frac{1}{2}$	+	+
DJ 60305	Dec. 29	do	6	+	+
DB 57572	1937 Jan. 1	do	6 $\frac{1}{2}$	+	+
DH 49013	Jan. 3	do	6 $\frac{1}{2}$	+	+
DS 45043	Jan. 4	do	7	+	+
DS 45046	Jan. 7	do	7	+	+
DS 38714	Jan. 14	do	7	+	+
DS 44824	Jan. 18	do	7	+	o
DH 80340	Jan. 19	Weak calf (died)	7	—	+
DS 15129	Jan. 23	Abortion	7	+	+
DH 80342	Jan. 23	do	7 $\frac{1}{2}$	+	+
DJ 30446	Jan. 24	Weak calf (died)	7 $\frac{1}{2}$	+	+
DA 55134	Jan. 27	Abortion	7	+	+
DS 94161	Feb. 1	do	7 $\frac{1}{2}$	+	+
DA 75159	Feb. 2	Weak calf (died)	8	+	+
DS 87944	Feb. 3	do	7	+	+
DA 55713	Feb. 4	Abortion	7	+	o
DH 30449	Feb. 8	do	8	+	+
DK 64886	Feb. 24	do	8	—	+
DH 30422	Mar. 6	do	7 $\frac{1}{2}$	+	+

GROUP 3.—CONTROLS (NOT FED PRODUCT)

DK 74131	1937 Jan. 8	Abortion	6	+	+
DK 74133	Jan. 8	do	5	+	+
DS 45048	Jan. 12	do	7	+	+
DS 87973	Jan. 13	do	5	+	+
DJ 24128	Jan. 15	do	7 $\frac{1}{2}$	+	+
DH 80367	Jan. 17	do	8	+	+
DS 910	Jan. 18	do	6	+	+
DS 94162	Jan. 20	do	6	+	+
DH 80344	Jan. 23	do	7 $\frac{1}{2}$	+	+
DJ 30420	Jan. 24	Weak calf (died)	7 $\frac{1}{2}$	+	+
DJ 30421	Jan. 25	Abortion	7 $\frac{1}{2}$	+	+
DS 72367	Feb. 4	do	6	+	o
DH 46912	Feb. 16	Weak calf (died)	8	+	+
DA 55724	do	Abortion	7 $\frac{1}{2}$	—	+
DJ 43492	Feb. 20	do	6	+	+
DJ 43491	Feb. 23	do	6 $\frac{1}{2}$	+	+
DK 64887	Feb. 27	do	7	+	+
DH 80348	Mar. 5	do	6 $\frac{1}{2}$	+	+
DH 76369	Mar. 6	do	7	+	+

¹ +, *Br. abortus* present; —, *Br. abortus* absent; o, not determined.

exposure only. However, the infection was present in uterine materials or colostrum, and in most cases in both of these substances, of all 58 cattle when their gestations terminated.

The first abortion occurred December 27, 1936, 29 days after the heifer was exposed to *Brucella abortus*. Abortions continued to occur until the early part of March 1937.

Of the 19 heifers in group 1, which was fed the product 48 days prior to and 27 days subsequent to exposure, all aborted. Of the 20 heifers in group 2, which received the product subsequent to exposure only, 16 aborted and 4 gave birth to weak calves that did not survive. Of the 19 control heifers in group 3, which did not receive the product, 17 aborted and 2 gave birth to weak calves that did not survive.

The results of this carefully controlled test indicate that the Crawford 3-V Tonic was ineffective in preventing pregnant heifers from becoming infected with Bang's disease or in preventing them from aborting after having acquired it.

BOWMAN'S PRODUCT

An analysis of the Bowman's product by the United States Food and Drug Administration showed it to be a light-brown granular material containing sugar (sucrose), 90.3 percent; invert sugar, 4.3 percent; wood creosote, 0.1 percent; ash, 1.5 percent; and moisture by difference. No other ingredients were detected.

In order to obtain definite information as to whether this product possesses value as a preventive or cure for Bang's disease or in causing the disappearance of *Brucella abortus* agglutinins from the blood serum of cattle which have acquired it, the following experiment was made.

EXPERIMENTAL PROCEDURE

Forty heifers were purchased from farms that were free from Bang's disease. As a result of pregnancy examinations, all the heifers were believed to be from 6 weeks to 4 months with calf. They gave negative results to the agglutination test for Bang's disease at the time they were purchased as well as after their delivery at the farm where the test was carried on. This farm was located a few miles from the University of Wisconsin and had been rented for making the test. Here the animals were divided into 2 groups of 20 each, similar as to condition. The Bowman's product was fed to the heifers in group 1 in accordance with the directions of the manufacturer. The heifers in group 2 were employed as controls, receiving none of the product. The Bowman's product fed to the experimental cattle was obtained indirectly by purchase in the original sealed packages, as the manufacturer of it was unwilling to cooperate in a test of the sort outlined and performed.

The 20 heifers in group 1 received four series of feedings of the product, consisting of four doses of 2 pounds each in accordance with the directions appearing on the labels of the packages. It was mixed with a small quantity of millfeed and given to the heifers in the morning before their regular feeding was commenced. It was consumed promptly by them.

The first series of feedings was on March 24, 25, 27, and 29, 1937. The feeding of the product was then discontinued for 1 week, when

the second series of feedings was commenced. These four feedings were administered on April 6, 8, 10, and 12. Feeding was again discontinued for a little more than 2 months, and the third series of feedings was given on June 19, 21, 23, and 25. After an interval of 30 days, the fourth series of feedings was given on July 23, 25, 27, and 29. Each heifer in group 1 thus received, during the four series of feedings, 16 doses of the product consisting of 2 pounds each, a total quantity of 32 pounds.

The 40 heifers in groups 1 and 2 were exposed to Bang's disease April 14, 1937, 2 days after the heifers in group 1 had received the second series of feedings. The *Brucella abortus* exposure of the cattle was made by representatives of the United States Bureau of Animal Industry and the University of Wisconsin. Use was made of the conjunctival method, which consisted in instilling a very small quantity of a suspension of *Br. abortus* in the mucous membrane of the eye. The suspension of *Br. abortus* used was prepared with three strains of the micro-organism, designated respectively, 213, 1927, and 214. Strain 213 had been isolated from the supramammary lymph glands of an infected cow about 14 months previously; strain 1927 had been isolated from an aborted fetus about 6 months previously; and strain 214 had been obtained about 6 months before from the milk of an infected cow. Three small drops of the suspension were deposited on the mucous membrane of one eye of each animal. Use was made of but 5 cc. of the suspension to expose the 40 heifers, each animal thus receiving 0.12 cc. of the material.

All the heifers gave negative results to the Bang's disease agglutination test on April 14, 1937, the day they were subjected to *Brucella abortus* exposure. On the next test, which was made April 20, 6 days later, one of the treated heifers and one of the controls gave a suspicious reaction to the test. The remaining heifers gave negative results. On the third test, which was made April 26, 12 days subsequent to their exposure, all the heifers reacted to the agglutination test in titers of from 1 to 50 to 1 to 400, there being but a slight difference between the intensity of the reactions of the treated heifers and the controls. The titers of the reactions given by the heifers in both groups gradually increased until they became 1 to 3,200 or higher.

When the gestation period of each heifer terminated, two guinea pigs were injected with a sample of her uterine material and four others with a composite sample of her colostrum for the purpose of determining whether *Brucella abortus* was present in these substances. In the event the heifer aborted, the fetus was cultured.

RESULTS OF THE EXPERIMENT

The results of the experiment (table 2) were obtained on 19 heifers that were fed the Bowman's product and 20 controls that did not receive it. One heifer that received the product was eliminated from the experiment before its completion because she was found to be nonpregnant.

The first abortion occurred May 12, 1937, 28 days subsequent to *Brucella abortus* exposure. The last of the 39 heifers completed her gestation period December 27, 1937.

Of the 19 heifers in group 1 which received eight doses of the Bowman's product previous to *Brucella abortus* exposure and eight doses

subsequent to exposure, 1 produced a vigorous calf, 1 a weak calf which lived, and 1 a weak calf which died. Sixteen heifers aborted. *Br. abortus* was demonstrated to be present in the uteri or colostrum, or both, of the 19 heifers when their pregnancies terminated and was isolated from the fetuses of 11 of the 16 heifers which aborted.

Of the 20 controls in group 2 which did not receive the product, 1 produced a vigorous calf and 4 others produced weak calves which died. Fifteen of the heifers in this group aborted. The presence of *Brucella abortus* was demonstrated in the uterine material or colostrum, or both, of all 20 heifers. The organism was isolated from the fetuses of 12 of the 15 heifers which aborted.

TABLE 2.—Results of feeding Bowman's product to pregnant heifers to prevent or cure Bang's disease

[In both groups, date of *Brucella abortus* exposure, Apr. 14, 1937. In group 1, date of first feeding, Mar. 24, and date of last feeding, July 20, 1937; number of feedings, 16]

GROUP 1.—ANIMALS FED PRODUCT

Animal designation	Date of termination of gestation	Outcome of pregnancy	Approximate stage of pregnancy	Results of examination for <i>Brucella abortus</i> ¹	
				Uterus	Colostrum
	1937		Months		
DS 76550	May 21	Abortion	6	+	+
DX 9903	May 25	do	4	+	+
EA 79101	May 28	do	4	+	+
DS 4806	June 6	do	3	+	+
DS 4923	June 7	do	4	+	+
DS 4924	June 20	do	3½	+	+
DS 76818	July 1	do	4	+	+
DX 9901	do	do	1	+	—
DS 76817	July 5	do	4	+	+
DS 4930	July 8	do	3½	+	+
DS 4926	July 12	Weak calf (lived)	4½	+	+
DS 4808	July 13	Abortion	4½	+	+
DS 4925	July 14	Weak calf (died)	5½	+	+
DS 4807	July 16	Abortion	4½	+	+
DS 4804	July 21	do	3½	+	+
DX 9947	July 26	do	4	+	+
DS 76554	July 27	do	3	+	+
DX 9918	Sept. 17	Vigorous calf	4	—	+
DS 4928	Aug. 26	Abortion	4½	+	+

GROUP 2.—CONTROLS (NOT FED PRODUCT)

	1937				
EA 67391	May 12	Abortion	5½	+	+
DH 95809	May 23	do	4	+	+
DH 80416	May 26	do	4½	+	+
EA 7914	May 29	do	4½	+	—
DB 99858	do	do	4	+	+
DJ 19847	May 31	do	4½	+	+
DS 42803	do	do	4	+	+
EA 31533	June 6	do	4	+	—
EA 67395	June 14	Weak calf (died)	5½	+	+
EA 31522	June 19	Abortion	4½	+	+
DJ 19848	June 28	Weak calf (died)	5	+	+
DJ 81035	July 1	Abortion	4	+	—
EA 31525	July 4	do	3½	+	+
EA 67392	July 20	do	3½	+	+
DX 9923	July 21	do	3½	+	+
EA 31524	Aug. 3	do	2½	+	+
DH 95810	Aug. 19	do	3½	+	+
DH 80417	Sept. 13	Weak calf (died)	3½	—	+
DH 80419	Aug. 19	Vigorous calf	3½	+	—
EA 31529	Dec. 27	Weak calf (died)	3	0	+

¹ +, *Br. abortus* present; —, *Br. abortus* absent; 0, not determined.

The results of the experiment, therefore, definitely show that the Bowman's product was ineffective either in the prevention or cure of Bang's disease. Each heifer in group 1 received eight 2-pound doses of the product before exposure to *Brucella abortus* and the same quantities after exposure. However, the animals exhibited no more resistance to the disease than the heifers in group 2 that did not receive the product.

INEFFECTIVENESS OF PRODUCT IN CAUSING DISAPPEARANCE OF BRUCELLA
ABORTUS AGGLUTININS FROM BLOOD OF CATTLE

To determine whether the Bowman's product is effective in causing the disappearance of *Brucella abortus* agglutinins from the blood serum of cattle reacting to the agglutination test for the disease, 10 of the heifers in group 1 and 10 of those in group 2 were retained for several months after their pregnancies had terminated and agglutination tests made of their blood serum at about monthly intervals. The results are shown in table 3.

TABLE 3.—Results of tests to determine the effectiveness of Bowman's product in reducing *Brucella abortus* agglutinins in the blood serum of cattle reacting to the agglutination test for Bang's disease

Group No. ¹	Blood titer on indicated number of days after exposure							
	70		98		119		148	
	Average	Minimum	Average	Minimum	Average	Minimum	Average	Minimum
1 (fed Bowman's)	1:2,510	1:800	1:4,080	1:800	1:6,560	1:400	1:23,200	1:3,200
2 (not fed Bowman's)	1:2,250	1:800	1:2,920	1:800	1:4,560	1:100	1:27,680	1:800

Group No.	Blood titer on indicated number of days after exposure					
	181		202		238	
	Average	Minimum	Average	Minimum	Average	Minimum
1 (fed Bowman's)	1:24,000	1:400	1:18,080	1:400	1:23,520	1:400
2 (not fed Bowman's)	1:18,440	1:400	1:10,280	1:400	1:14,200	1:400

¹ 10 animals in each group.

The Bowman's product was found to be ineffective in causing cattle reacting to the Bang's disease agglutination test to become nonreactors to the test or in otherwise having any demonstrable effect on the *Brucella abortus* agglutinin concentration in the blood serum of cattle affected with the disease. As shown by table 3, about 8 months after the two groups of animals were infected with the disease, the average agglutination titer of the blood serum of the group which had received 32 pounds of Bowman's product was higher than that of the group which had not received it.

SUMMARY AND CONCLUSIONS

In work on Bang's disease and possible methods for its control, Federal and State experiment stations have studied the effect of various drugs and chemical substances. In every instance the drug or chemical tested has given negative results, but in spite of these findings and the wide publicity given them, so-called remedies for Bang's disease still appear on the market, and farmers continue to spend large sums for them.

The present study, conducted under Congressional authority, consisted of carefully controlled tests of two widely used, alleged remedies for Bang's disease, "3-V Tonic" and "Bowman's."

In the testing of 3-V Tonic, a group of 19 pregnant heifers, negative to the agglutination test, was fed this preparation prior to exposure to virulent strains of *Brucella abortus*; a second group of 20 heifers was fed prior and subsequent to exposure; and a third group of 19 heifers, as controls, received only exposure. All heifers in group 1 aborted; 16 heifers in group 2 aborted and 4 gave birth to weak calves; 17 heifers in the control group aborted and 2 gave birth to weak calves. *Brucella abortus* was recovered from uterine material or colostrum, and in most cases from both of these substances, in all 58 animals following parturition.

In the testing of Bowman's product, a group of 19 pregnant heifers, negative to the agglutination test, was fed this preparation prior and subsequent to exposure to virulent strains of *Brucella abortus*; a second group of 20 heifers, as controls, received only exposure. Of the 19 heifers in group 1, 16 aborted, 1 produced a weak calf which died, 1 a weak calf which survived, and 1 a vigorous calf. Of the 20 control animals, 15 aborted, 4 each produced a weak calf which died and 1 a vigorous calf. *Brucella abortus* was recovered from uterine material or colostrum, or both, in each of the 39 heifers following parturition. Continued testing of the blood titer of 10 heifers in group 1 and 10 heifers in group 2, for a period of 8 months after exposure, showed that the feeding of Bowman's had no effect on reducing the blood titer following infection.

The 3-V Tonic and Bowman's product were thus found to be ineffective in preventing or curing Bang's disease. These results, together with previous negative findings obtained from other drugs and chemical substances, indicate that, in the light of present scientific knowledge, such products do not provide protection against the disease.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 60

WASHINGTON, D. C., MAY 1, 1940

No. 9.

NEW PHYSIOLOGIC RACES OF FLAX RUST

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INTRODUCTION

The results obtained from investigations of physiologic specialization in flax rust (*Melampsora lini* (Pers.) Lév.) during the years 1935 to 1938, inclusive, have rendered inadequate the key for the identification of races presented by the writer in an earlier paper (1)³ and have vitiated some of the conclusions concerning the prospects for developing rust-immune varieties of flax through hybridization. The varieties of common flax (*Linum usitatissimum* L.) formerly reported as immune from all races of flax rust have been found to be susceptible to one or more of the races collected since 1935. Consequently, the number of flax varieties giving a differential reaction to the physiologic races of flax rust (*M. lini*) has been greatly enlarged. The results of investigations made since 1935 are reported in this paper.

MATERIAL AND METHODS

Methods of obtaining and propagating collections of flax rust, of inoculating and incubating flax plants, and of classifying host reactions and types of infection were described by the writer in 1935 (1). All results reported were obtained on flax plants approximately 30 days old, grown in the greenhouse at Fargo, N. Dak., during the winter months November to March, inclusive. The greenhouse was kept at a temperature of about 14° C. at night and 18° to 20° during the day. A light day of 16 hours was maintained during the period of pustule formation by supplementing daylight with artificial illumination from 200-watt Mazda bulbs. Final readings of the classes of host reaction and types of rust infection were made 10 to 15 days after inoculation, depending upon the effect of temperature and light on the rapidity of pustule development.

EXPERIMENTAL RESULTS

NEW DIFFERENTIAL VARIETIES OF FLAX

In order to differentiate the races of *Melampsora lini* collected since 1935, it has been necessary to add three varieties, Argentine (C. I. 462),⁴ Bombay (C. I. 42), and Ottawa 770 B (C. I. 355), to the former list of differential varieties. These three varieties have been either immune from or susceptible to every race of rust thus far isolated, under all conditions encountered in the greenhouse.

¹ Received for publication February 7, 1940. Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the North Dakota Agricultural Experiment Station.

² The writer is indebted to A. C. Dillman, associate agronomist, Division of Cereal Crops and Diseases, for supplying seed of 150 or more varieties, for the preliminary grouping of varieties as shown in the tables, and for a critical reading of the manuscript. The writer is also indebted to A. C. Arny, associate agronomist, Minnesota Agricultural Experiment Station, and to José Vallega, of the Instituto Fitotécnico, Llavallol, Argentina, for supplying seed of a number of varieties.

³ Italic numbers in parentheses refer to Literature Cited, p. 591.

⁴ C. I. refers to accession number of the Division of Cereal Crops and Diseases.

The original strain of Williston Brown (C. I. 803) was lost. This strain was susceptible to wilt (*Fusarium lini* Bolley) and produced little seed even under favorable climatic conditions, so that no reserve of seed was accumulated. However, several single-plant selections from this strain were grown in the greenhouse during the winter of 1935-36 and from these a strain was secured that has considerable wilt resistance in addition to a more resistant reaction to the races of rust that were differentiated by the original strain of Williston Brown. This new strain (C. I. 803-1) was substituted for the former one. It has the necrotic (type 2) reaction to races to which it is resistant, and is not completely susceptible to many of the races collected in North America. The (type 3) uredia developed normally until they began to produce spores. After 2 or 3 days, development stopped, the pustules ceased to sporulate, and the leaves dried up. To other races it was highly susceptible; the uredia produced spores profusely for a period of 10 days to 2 weeks.

VARIABILITY IN REACTION OF DIFFERENTIAL VARIETIES OF FLAX

It has been customary in classifying rust reaction of crop plants growing under field conditions to take into consideration both the type and the number of pustules. Myers (6) and Vallega (9) followed this method in their classifications of the rust reactions of flax varieties. However, Hart (2) has shown that environmental conditions such as the temperature during the period the plants are in the moist chamber, the temperature during the remainder of the incubation period, and any condition that affects the vigor of the host plant, such as light intensity, temperature, moisture, and mineral nutrition, may alter not only the type but also the number of pustules. These conclusions of Hart have been verified repeatedly in studies made by the writer. In addition, it was observed that apparently identical changes in environmental conditions produced variable responses in different varieties and that the amount and viability of the inoculum affected the reaction of some varieties. The impracticability of accurately controlling the amount of inoculum, and the difficulties in spreading the spores evenly over all the inoculated areas and of rigidly controlling all phases of the environment to which the plant is subjected following inoculation render an attempt to classify degrees of resistance and susceptibility ineffectual. Attempts to differentiate too finely between degrees of resistance and susceptibility may lead to confusion and to a misunderstanding of results obtained at different localities or under variable conditions. A record of observations made on the reactions of the differential varieties when grown under a range of seasonal conditions in the greenhouse at Fargo may assist in the interpretation of results obtained in other localities.

The data presented in this paper were obtained during the winter months under the conditions previously described. However, the reaction range of the differential varieties to races of *Melampsora lini* collected locally has been studied throughout the year. On the basis of these studies with the 24 races of *M. lini* thus far differentiated, the differential varieties have been divided into 4 groups according to the stability of their general reaction under varying conditions and the range of their reaction to given races. These groups are as follows: (1) Stable reaction, always either immune or susceptible; (2) slightly variable reaction, immune, resistant, or susceptible; (3) slightly vari-

able reaction, a wide and almost continuous reaction range with many intermediates; and (4) variable and often intermediate reaction, a wide reaction range with certain races.

The differential varieties classed as having stable reaction have been either immune from or susceptible to every race of rust thus far isolated under all conditions and consequently have made ideal differentials. These varieties are J. W. S.⁵ (C. I. 708-1) and three varieties, namely, Argentine (C. I. 462), Bombay (C. I. 42), and Ottawa 770 B (C. I. 355), that have been added to the list of varietal testers in order to differentiate the new physiologic races of rust.

The three varieties placed in the group having a slightly variable reaction have been immune, resistant, or susceptible to each of the 24 races of flax rust and have been satisfactory as differentials under all seasonal conditions. These varieties, Abyssinian (C. I. 701), Akmolinsk (C. I. 515-1), and Williston Golden (C. I. 25-1), have the chlorotic type of resistance (type 1) in which the uredia are minute to small and scattered in chlorotic areas of the leaves with but little necrosis of adjacent tissue. The distinction between susceptible and resistant-to-immune reaction in Abyssinian and Akmolinsk has been so wide under all conditions that there has been no difficulty in classifying their reaction. Under very favorable growing conditions for flax, the resistant-minus reaction (type 1 to 1+) of Williston Golden approached a susceptible-minus reaction (type 1+ to 3) but did not approximate it closely enough to be confusing.

Buda (C. I. 270-1), the only differential variety placed in the third group, had the most diverse reactions of all varieties tested. Its reaction range extended from immunity, through most of the intermediate stages, to a high degree of susceptibility. However, its range of reaction to any single race of rust was relatively narrow. Under adverse conditions for growth of flax the infection type on Buda sometimes approached that next lower in the scale of susceptibility. When conditions were such that the plants were growing thriftily, no difficulty due to seasonal conditions was encountered in identifying races differentiated by Buda.

The differential varieties in the fourth group had, to many races of rust, an intermediate reaction that was sensitive to changes in environmental conditions, so that considerable care was necessary in differentiating between degrees of resistance or susceptibility. To the races that they differentiated, these varieties had a relatively stable reaction. Two of these varieties, Kenya (C. I. 709-1) and Williston Brown (C. I. 803-1), had the necrotic type of resistance (type 2), in which the inoculated areas of the leaves became necrotic before the uredia were fully developed and the incompletely developed pustules were usually aggregated near the margins of necrotic areas. Isolated pustules were usually well developed but became prematurely surrounded by a necrotic zone. The reaction of "pale blue crimped"⁶ (C. I. 647) to races to which it had an intermediate infection type was peculiar in that this variety became more resistant when growing vigorously under favorable conditions and more susceptible when weakened or retarded by unfavorable growing conditions.

The reaction of Argentine (C. I. 705) to races 1 to 14, inclusive, has been tabulated (1); but, since this variety had the variable reaction

⁵ J. W. S. are the initials of J. W. Stewart, the originator of the variety.

⁶ The word "crimped" is here used to describe the petal margins, which are incurved and somewhat wavy.

of group 4 and since it has not been necessary to use it in the key for the identification of physiologic races, it has been dropped from the list of differential varieties.

NEW PHYSIOLOGIC RACES OF FLAX RUST

Ten physiologic races of flax rust, in addition to the 14 previously described by the writer (1), have been differentiated by the type of infection produced on 11 varieties of cultivated flax.⁷ All varieties that have been tested, including many that formerly were considered immune from flax rust (1, 3, 4, 6), have been found susceptible to 1 or more of the races isolated since 1935. A revised key to the races of *Melampsora lini* follows.

KEY

Buda resistant:		Buda susceptible:	
Williston Golden resistant:		Williston Golden resistant:	
Akmolinsk resistant:	Race	Akmolinsk resistant:	
Williston Brown resistant:	10	J. W. S. resistant:	Race
Williston Brown susceptible:	1	Kenya resistant:	4
Akmolinsk susceptible:		Kenya semiresistant:	12
J. W. S. resistant:	5	J. W. S. susceptible:	13
J. W. S. susceptible:	7	Akmolinsk susceptible:	
Williston Golden susceptible:		J. W. S. resistant:	8
Akmolinsk resistant:		J. W. S. susceptible:	16
"Pale blue crimped" resistant:	11	Williston Golden susceptible:	
"Pale blue crimped" susceptible:	6	Akmolinsk resistant:	
Akmolinsk susceptible:	20	J. W. S. resistant:	
Buda semiresistant:		Bombay resistant:	2
Williston Golden resistant:		Bombay susceptible:	24
Akmolinsk resistant:		J. W. S. susceptible:	9
J. W. S. resistant:	17	Akmolinsk susceptible:	
J. W. S. susceptible:	15	J. W. S. resistant:	
Akmolinsk susceptible:		Ottawa 770 B resistant:	19
Abyssinian resistant:	3	Ottawa 770 B susceptible:	22
Abyssinian susceptible:	18	J. W. S. susceptible:	21
Williston Golden susceptible:			
Akmolinsk resistant:	14		
Akmolinsk susceptible:	23		

The characteristic reaction of seedling plants of 11 differential flax varieties inoculated with 24 physiologic races of *Melampsora lini* and grown in the greenhouse during the winter months at Fargo, N. Dak., is given in table 1.

It will be noted in table 1 that several of the races of flax rust isolated since 1935 have a wider varietal range than any of the 14 races previously described (1). The races of rust having greater virulence, as measured in terms of the number of differential varieties on which they produced a susceptible reaction, were obtained from Minnesota, North Dakota, Oregon, and South America.

Three of the six newly differentiated races of flax rust, collected in Minnesota or North Dakota, were more virulent than any of the races previously collected in this area. Race 16 was the first one isolated to which Buda, Akmolinsk, and J. W. S. were all susceptible and the first race collected in this area to which Abyssinian was not resistant.

⁷ Since the preparation of this manuscript, two reports of work done by Straib in Germany have been published (7, 8). Straib differentiates eight physiologic races apparently different from those previously described by the writer. While several of his new races resemble somewhat certain races described in the present paper, the exact interrelations of these cannot be determined at present since he varied the technique employed by the writer and used additional host testers.

TABLE 1.—Reaction of 11 differential varieties of *Linum usitatissimum* to 24 physiologic races of *Melampsora lini*

Variety	C. L. No.	Reaction ¹ to physiologic race No.—																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Buda.....	270-1	R+	S-	SR	S	R+	R+	I	S	S-	R+	R+	S-	S-	SR	SR	R+	SR	R-	S	I	S	S	S	S
Williston Golden.....	25-1	R	S	R+	R+	R-	R+	R	R+	S-	S	S	R-	R-	S	R	R	R	R	S	S	S	S	S	S
Williston Brown.....	803-1	S	R+	S	S	S	S	R	S	R+	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S
Akmalinsk.....	515-1	I	R+	S	S	I	I	S	S	R+	I	I	S	S	I	S	S	S	S	S	S	S	S	S	S
J. W. S.....	708-1	I	S	S	S	S	S	S	S	S	I	I	S	S	S	S	S	S	S	S	S	S	S	S	S
"Pale blue crimped".....	647	S	S	S	S	S	S	S	S	S	I	I	S	S	S	S	S	S	S	S	S	S	S	S	S
Kenya.....	709-1	R+	S	SR	S	SR	R	S	S	R	I	I	S	S	R+	S	S	R	S	S	S	S	S	S	S
Abyssinian.....	701	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Argentine.....	462	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Ottawa 770 B.....	355	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Bombay.....	42	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I

¹ Plus and minus signs indicate somewhat greater or less resistance or susceptibility than the letter designating the host reaction. The letters signify the following: I, Immune; R, resistant; SR, semiresistant; and S, susceptible.

Race 23 was the only one collected in the midwest seed-flax-producing area to which both Williston Golden and Akmolinsk were susceptible. Bombay, which had been immune from every rust collection previously tested, was susceptible to race 24, collected in North Dakota in 1938. Races 15, 17, and 18 had only minor differences in reaction from those previously described. Both Buda and Williston Golden were more resistant to race 15 than to race 9. The resistance of Akmolinsk differentiated race 17 from race 3. The moderate susceptibility of Abyssinian to race 18 differentiated this race from race 3.

The single uredial collection obtained from Oregon, designated race 21, produced a susceptible or semiresistant reaction on all the differential varieties previously used (1). The three added differentials, Argentine, Bombay, and Ottawa 770 B, were immune from race 21. This was the only North American race, with the exception of race 8, collected in Oregon in 1934, to which Abyssinian was highly susceptible.

Physiologic races 19, 20, and 22, obtained only from South America, possessed infection potentialities distinct from all North American races. Every variety used in differentiating physiologic races of flax rust was either highly susceptible to or immune from these South American races. The Argentine flaxes, formerly considered immune from rust (1, 2, 3, 4, 6), were highly susceptible to races 19, 20, and 22. Bombay, J. W. S., and Ottawa 770 B were immune from race 19; Bombay, Buda, J. W. S., and Ottawa 770 B were immune from race 20; and Bombay and J. W. S. were immune from race 22. The fact that race 22 was found able to attack Ottawa 770 B, which is immune from all other races, is of special importance, as previously this variety had been used as a rust-immune parent in breeding immune varieties of flax in the United States (4). Bombay and J. W. S. were found to be immune from all three races of rust from South America. However, Bombay was susceptible to race 24, from North Dakota, and J. W. S. was susceptible to several other North American races.

Buda was previously reported (1) resistant to race 7, but in subsequent tests has been consistently immune, and it is so classified in table 1.

GEOGRAPHIC DISTRIBUTION OF RACES OF FLAX RUST

Although no systematic survey has been made for the collection and identification of physiologic races of *Melampsora lini*, determinations have been made of collections from various sources. During the 4-year period from 1935 to 1938, 81 race determinations were made in North American urediospore collections. In addition, race determinations were made on two collections of viable telia obtained from South America. Germinating teliospores were used to inoculate flax plants and aecia were developed. A susceptible variety, Bison (C. I. 389), was inoculated with aeciospores from a single aecium, and race determinations were then made by using the urediospores thus produced to inoculate the differential varieties. From one of these telial collections two physiologic races were identified, and from the other collection four physiologic races were identified.

Physiologic races 1 to 18, inclusive, and 21, 23, and 24 were obtained from different localities in North America, and races 19, 20, and 22 were obtained from South America. The distribution of these races and the number of times each race was collected in each location are shown in table 2.

TABLE 2.—Geographic distribution of physiologic races of *Melampsoora lini* and number of times each race was identified in collections, 1935–38

Year and source of collection	Isolates of physiologic race No. —																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1935:	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
Canada ¹	1																							
Minnesota.....			3	3														3						
North Dakota.....	4		2	1				1				2					10		1	1				
Uruguay ²																								
1936:																								
Minnesota.....	2		2	2													1							
North Dakota.....			1																		1			
Oregon ³																								
1937:																								
Argentina ⁴			1																					
Minnesota.....	4		3			4	6					1			5		1	1		1		1		
North Dakota.....	2		1			1	1							3			1							
1938:																								
North Dakota.....	1	1													1									1
Texas ⁵																								
Total.....	14	1	13	6	5	7	0	0	1	0	0	3	0	10	1	1	16	1	2	2	1	1	1	1

¹ Collected by W. G. McGregor at Ottawa, Ontario, Canada.² Collected by J. G. Fischer and Albert Boerger, La Estanzuela, Colonia, Uruguay.³ Collected by B. B. Robinson at Astoria, Oreg.⁴ Collected by José Vallega, Llavallol, Argentina.⁵ Collected by E. S. McFadden, at Victoria, Tex.

There appeared to have been little change since the earlier report (1) in the races of rust that predominated in the midwest seed-flax-producing area. The ones with a more limited varietal range continued to be predominant, although there were races present that had a wider varietal host range. Since the principal commercial seed-flax variety, Bison, is susceptible to all known races of flax rust, there probably was no natural selective survival going on among the more widely virulent strains, such as might take place if a variety were grown that was resistant to the present prevailing races but susceptible to those races having a wider varietal range.

Races 19 and 20 were segregated from a telial collection obtained from Uruguay. These 2 races also predominated in the aecial isolates made from the telial collection received from Argentina, and races 3 and 22 also were obtained from these latter isolates. Races 19, 20, and 22 were obtained only from South America and therefore special care was taken to prevent their escape from the greenhouse. Tests were conducted only during the winter months and all plant parts, pots, and soil were steamed before being discarded.

In 1938, a uredial collection sent from a Texas flax field sown with Bison seed obtained from North Dakota was identified as race 14. This race was prevalent in Minnesota and North Dakota flax fields the preceding year and the Texas infection probably originated from telia sown with the seed.

Race 24 was isolated from a row of heavily rusted Bombay sown in the field at Fargo, N. Dak., in 1938. This seed was obtained from José Vallega, who reported this variety immune in Argentina (9).

REACTION OF VARIETIES OF FLAX

One of the objects of a study of physiologic specialization in parasitic organisms is to facilitate the production of immune or resistant varieties of crop plants. A knowledge of the regional and world-wide variation of the parental material to the disease-producing organism is essential, especially when the highly specialized rusts are involved. In the data presented in an earlier paper (1), based on a study of North American collections of *Melampsora lini*, so few of the flax varieties showed differential response to the physiologic races then isolated that it was considered more desirable, in recording the reaction of varieties, to list them according to type of flax rather than according to their response to the different races of flax rust. The varietal specificity of several of the more recently isolated races of flax rust has made it desirable to group the flax varieties on the basis of their differential reaction to the races of rust thus far isolated. On this basis the 201 varieties and strains of flax that have been tested with the 16 principal physiologic races, Nos. 1, 2, 3, 4, 5, 7, 8, 9, 10, 16, 19, 20, 21, 22, 23, and 24, have been grouped as follows: (1) Varieties and strains susceptible to all these races; (2) those having the specific immunity of (a) Argentine, (b) Ottawa 770 B, (c) J. W. S., and (d) Bombay; (3) those having varying degrees of resistance and susceptibility; and (4) those too heterogeneous to classify as to predominant type of reaction. The several races that were not included have pathogenic properties differing but slightly from one or more of those used in these tests and the additional information would not have warranted the increased cost in time and effort their use would have entailed. Cases where the variety was not pure for rust reaction and instances

where a variety consistently exhibited an intermediate type of reaction have been noted.

The varieties and strains of flax predominantly susceptible to all 16 physiologic races of *Melampsora lini* that were used are listed in the following tabulation. Of the 201 varieties and strains of flax tested, 91 were in this group.

Varieties of flax susceptible to physiologic races 1, 2, 3, 4, 5, 7, 8, 9, 10, 16, 19, 20, 21, 22, 23, and 24 of Melampsora lini and immune from none

Group and variety	C. I. No.	Group and variety	C. I. No.
Dehiscent flax (<i>Linum usitatissimum crepitans</i>):		Seed flax—Continued.	
From Siberia.....	295	Petals broad, flat—Continued.	
From Germany.....	469	American and European—Con.	
From Ukraine.....	506	Pink-flowered:	
Do.....	507	Deep pink.....	648
Seed flax (<i>Linum usitatissimum</i>):		Long No. 4.....	400
Petals broad, flat:		Long No. 66.....	337
Abyssinian:		Do.....	719
Abyssinian Yellow-seed.....	36	Long No. 83.....	354
Abyssinian.....	300	Pale pink.....	² 173-1
Do.....	¹ 302	Do.....	² 173-3
Abyssinian from Egypt.....	¹ 380	Pale pink (M25-228).....	479
Abyssinian from Kenya,		Tall pink.....	451-3
East Africa.....	707	Tammes pink.....	334
American and European:		Tammes pink, type 8.....	772
Blue-flowered:		Tammes deep pink.....	336
Bison.....	389	Tammes deep pink,	
Bolley No. 32 1823.....	² 754	type 9.....	¹ 773
Buda.....	326	Argentine:	
Linota.....	244	Capa.....	³ 720
Minnesota 25-410.....	421	De Sanare.....	³ 1002
Minnesota 25-202.....	447	Klein 10 e.....	³ 887
Minnesota 25-221.....	423	Rosario.....	³ 316
Minnesota 25-245.....	446	Indian:	
Minnesota 25-107.....	438	Howard and Khan (5):	
Minnesota 25-241.....	458	var. <i>luteum</i> , type 1.....	
North Dakota Resist-		var. <i>cyaneum</i> , type 8.....	
ant 5.....	411	var. <i>purpureum</i> , type	
North Dakota Resist-		11.....	
ant 52.....	275	var. <i>album</i> , type 15.....	(⁴)
North Dakota Resist-		var. <i>agreste</i> , type 22.....	
ant 114.....	489	var. <i>meridionale</i> , type	
North Dakota Resist-		25.....	
ant 714.....	399	var. <i>pratense</i> , type 28.....	
North Dakota Resist-		Russian:	
ant 726.....	412	Billings.....	³ 184
North Dakota 40016.....	428	Crimean (No. 2237) ⁵	³ 563
Pale blue.....	387-1	Fergana (No. 401) ⁵	512
Redwing.....	320	Novelty.....	³ 140
Slope.....	274	Samarkand (No. 2238) ⁵	³ 514
Winona.....	481	Hybrid:	
Argentine X Saginaw.....	660	Petals narrow, margins	
White-flowered:		incurved or crimped:	
Blanc.....	323-3	Crimped white (Minn.	
Diadem.....	³ 321	25-125).....	392
Ottawa white-flowered.....	24	Crimped white (Minn.	
Tammes white.....	329	29-55).....	⁶ 685
Tammes white, type 10.....	774	Ottawa 829-C.....	391
Tammes yellow seed.....	331	Pale blue.....	³ 646
Tammes yellow seed,		Tammes crimped white.....	330
type 13.....	777	Tammes crimped white,	
		type 11.....	775

See footnotes at end of table.

Varieties of flax susceptible to physiologic races 1, 2, 3, 4, 5, 7, 8, 9, 10, 16, 19, 20, 21, 22, 23, and 24 of *Melampsora lini* and immune from none—Continued

Group and variety	C. I. No.	Group and variety	C. I. No.
Seed flax—Continued.		Fiber flax—Continued.	
Petals broad, flat—Con.		Blue-flowered—Con.	
Hybrid—Continued.		Liral Crown.....	882
Petals narrow, etc.—Con.		Peerless.....	695
Tammes type 12 (seeds brown).....	776	Saginaw.....	449
Yellow seed B.....	325	Stormont Cirrus.....	881
Fiber flax (<i>Linum usitatissimum</i>):		Stormont Gossamer.....	³ 883
Blue-flowered:		White-flowered:	
Althausen.....	628	Blenda.....	
Do.....	¹ 630	Concurrent.....	801
Dalgonetz.....	498	Friesland white.....	56-1
F. I. ² No. 3.....	³ 694	Minnesota 25-64.....	420
Hercules.....		Pinnacle.....	693
J. W. S.....	¹ 388	Saginaw white.....	448

¹ Some plants resistant to or immune from each race.

² Susceptible (S-) to most North American races. S- denotes a susceptible-minus host reaction in which the uredia are large but less abundant than in a susceptible reaction and in which there is considerable distortion and chlorosis or necrosis of the infected leaf tissues.

³ Some plants resistant to or immune from North American races.

⁴ Susceptible (S-) to race 10.

⁵ Obtained from Dr. N. I. Vavilov, Union of Soviet Socialist Republics, in March 1930.

⁶ Some plants immune from all races except race 22.

⁷ F. I. = Fiber investigations.

While there was no absolute correlation between flax type and rust reaction, there appeared to be definite relationships. The four strains of dehiscant flax (*Linum usitatissimum* var. *crepitans* Bönningh.) were uniformly susceptible. Most American and European seed flax varieties, as well as most fiber flax varieties, were susceptible to all races of rust. However, there were a few varieties in these groups that possessed resistance to or immunity from certain races. Several varieties of Abyssinian and of Indian type were highly susceptible, as also were a number of varieties having narrow incurved or crimped petals. Most of the Argentine and Russian flaxes listed as predominantly susceptible were not pure for rust reaction and contained varying percentages of plants immune from North American races but susceptible to races 19, 20, and 22 from South America.

It is possible that some of the varieties listed as susceptible to all races may give a differential reaction when tested with additional races of rust. No European rust collection has been tested, but the report by Henry (3) that flax varieties resistant or immune in The Netherlands were susceptible in Minnesota would indicate the existence of varieties susceptible to North American races but resistant to or immune from some European races. The variety Rosario (C. I. 316) and two selections of "pale pink" (C. I. 173-1 and 173-3) that formerly were reported (1) as resistant to North American races have been found to be somewhat susceptible and have been included among the susceptible varieties listed in the tabulation on pages 583-584.

It has been pointed out that four of the differential varieties, Argentine, Bombay, J. W. S., and Ottawa 770 B, have been either immune from or susceptible to each of the 24 races of flax rust. The reaction of these varieties suggests that each possesses a factor or factors for immunity from certain races of rust not common to the other three varieties. The studies that have been made on the inheritance of immunity from rust in flax bear this out. Henry (4) found that immunity from races of North American rust was dominant

and conditioned by a single factor in the varieties Bombay and Ottawa 770 B and by two factors in a selection of Argentine type. Myers (6) verified Henry's results in regard to Ottawa 770 B. He also found that the immunity of an Argentine flax (C. I. 712) could be explained by assuming the existence of two dominant factors governing rust reaction, one factor determining immunity and the other near-immunity from a North American rust collection. Unpublished data obtained by the writer have shown that immunity in J. W. S. is conditioned by a single dominant factor. Flax varieties having rust reactions similar to that of each of the differential varieties Argentine, Ottawa 770 B, J. W. S., and Bombay have been grouped in the following tabulations to facilitate inventories of potential parental material for breeding rust-immune flax.

Flaxes of Argentine type were immune from all North American rust collections but highly susceptible to races 19, 20, and 22 from South America. Varieties having either or both of the factors for immunity or near-immunity from North American rusts as found by Henry (4) and Myers (6) are in this group. Most of these varieties are definitely of the Argentine type or hybrids in which an Argentine type of flax was a parent. However, three pink-flowered American seed flaxes, one Indian, three Mediterranean, and two Russian type flaxes had the rust reaction characteristic of Argentine type varieties. Varieties and strains having the reaction of the Argentine type flaxes are listed in the following tabulation.

Varieties of seed flax (Linum usitatissimum) susceptible to physiologic races 19, 20, and 22 of Melampsora lini, and immune from races 1, 2, 3, 4, 5, 7, 8, 9, 10, 16, 21, 23, and 24

Group and variety	C. I. No.	Group and variety	C. I. No.
American and European:		Indian:	
Pink-flowered:		Howard and Khan (5):	
Bolley Golden.....	¹ 644	var. <i>pulchrum</i> , type 34.....	—
Bolley No. 32-1822.....	750	Mediterranean:	
Smoky Golden.....	751	Beladi.....	⁶ 377
Argentine:		Giza.....	¹ 378
Argentine (N. Dak. 1742) ..	¹ 342	Morocco.....	² 376-2
Argentine (Minn. 25-343) ..	² 417	Russian:	
Argentine (Minn. 25-341) ..	462	Damont.....	3
Argentine (Minn. 25-362) ..	472	Newland.....	² 188
Argentine (Minn. 25-330) ..	¹ 690	Hybrid:	
Argentine (Minn. 25-361-1) ..	¹ 691	Argentine × Saginaw.....	651
Argentine (Selection C. I.		Do.....	652
379-3).....	² 692	Do.....	653
Argentine (Minn. 25-323) ..	² 705	Do.....	⁷ 654
Biglow.....	² 414	Do.....	656
Capa 10.....	³ 721	Do.....	657
Capa 11.....	722	Do.....	⁷ 658
Kenya.....	⁴ 706	Do.....	⁷ 661
Do.....	² 709	Do.....	664
Light Mauve.....	⁵ 379-1	Do.....	667
Lineta Z 195.....	⁶ 1003	Bolley 37-5310.....	877
Lino Grande.....	¹ 381-2	Bolley 37-5242.....	878
Long No. 5.....	² 466	Natural hybrid, Rosario.....	820
Malabrigo.....	¹ 346	Selection Bison × (160 ×	
Do.....	696	179).....	818
Rio.....	280	Selection (19 × 112) × 19..	819

See footnotes at end of table.

Varieties of seed flax (Linum usitatissimum) susceptible to physiologic races 19, 20, and 22 of Melampsora lini, and immune from races 1, 2, 3, 4, 5, 7, 8, 9, 10, 16, 21, 23, and 24—Continued

Group and variety	C. I. No.	Group and variety	C. I. No.
Hybrid—Continued.		Hybrid—Continued.	
Selection of C. I. 385 (19 × 112).....	823	Selection of C. I. 385 (19 × 112).....	825
Selection of C. I. 496 (160 × 179).....	824	Walsh.....	645

¹ Some plants resistant to North American races.

² Some plants resistant or susceptible to North American races.

³ Resistant to races 16, 21, and 23.

⁴ Some plants resistant to all races except 22.

⁵ Resistant to North American races.

⁶ Some plants immune from races 19 and 20.

⁷ Some plants susceptible to North American races.

The high proportion of varieties having the Argentine type of rust reaction, but not pure for immunity from North American races of flax rust, may be due (1) to a relatively high percentage of natural crossing in these varieties or (2) to an immune and to a near-immune or resistant factor for rust reaction, as pointed out by Myers (6).

The varieties with the reaction of Ottawa 770 B, i. e., immune from all races except race 22 from South America, are listed in the following tabulation.

Varieties of seed flax (Linum usitatissimum) susceptible to physiologic race 22 of Melampsora lini, and immune from races 1, 2, 3, 4, 5, 7, 8, 9, 10, 16, 17, 18, 19, 20, 21, 23, and 24

Group and variety	C. I. No.	Group and variety	C. I. No.
Petals broad, flat:		Petals narrow, etc.—Continued.	
Argentine:		Minnesota hybrids—Continued.	
Bolley 37-5012.....	¹ 874	Saginaw × Ottawa 770 B—Continued.	
Klein 11-o.....	1004	Do.....	682
Lineta Z 176.....	885	Do.....	684
Pergamino selection.....	884	Do.....	685-1
Tammes pale blue.....	¹ 333	Do.....	686
Petals narrow, margins incurved or crimped:		Do.....	687
Ottawa 770 B.....	355	Do.....	² 681
Minnesota hybrids:		Winona × Ottawa 770 B....	672
Saginaw × Ottawa 770 B....	675	Do.....	673
Do.....	676	Do.....	674
Do.....	677	Do.....	716
Do.....	679		

¹ Some plants susceptible to all races.

² Some plants susceptible to races 19 and 20.

Most of these varieties were hybrids in which Ottawa 770 B was a parent. The origin of Tammes pale blue and of the four varieties from Argentina having rust reaction similar to that of Ottawa 770 B was not available.

The four varieties that were immune from the same physiologic races as the differential selection of J. W. S. are listed in the following tabulation.

Varieties of flax (Linum usitatissimum) susceptible to physiologic races 7, 9, 16, and 21 of Melampsora lini, and immune from races 1, 2, 3, 4, 5, 8, 10, 19, 20, 22, 23, and 24

Group and variety	C. I. No.	Group and variety	C. I. No.
Seed flax:		Fiber flax:	
Petals broad, flat:		Blue-flowered:	
Italia Roma.....	1 1005	J. W. S.....	708-1
Tammes light blue.....	332		
Tammes light blue, type 2..	766		

¹ Not highly susceptible to races 7, 9, 16, and 21.

Tammes light blue types were identical and pure for rust reaction. Italia Roma (C. I. 1005), in addition to being immune from the races to which the J. W. S. selection and Tammes light blue types were immune, was somewhat resistant to the races to which these varieties were susceptible. The reaction of Italia Roma suggested that it had a modifying factor for resistance in addition to the factor for immunity possessed by the differential strain of J. W. S.

The eight varieties listed in the following tabulation had previously been considered immune from all races of flax rust but were susceptible to race 24 found in field plots at Fargo, N. Dak., in 1938. These are all Indian types or hybrids in which an Indian type flax was a parent. The 12 varieties listed in the following tabulation and in that immediately preceding were the only varieties tested that were not predominantly susceptible to race 22 from South America.

Varieties of seed flax (Linum usitatissimum) susceptible to race 24 of Melampsora lini, and immune from races 1, 2, 3, 4, 5, 7, 8, 9, 10, 16, 19, 20, 21, 22, and 23.

Group and variety	C. I. No.	Group and variety	C. I. No.
Indian:		Hybrid:	
Howard and Khan (5):		Saginaw × Bombay.....	671
var. <i>minor</i> , type 29 ---		Vallega 2181-1.....	
var. <i>commune</i> , type 46 ---			
var. <i>commune</i> , type 48 ---			
var. <i>commune</i> , type 53 ---			
Bombay.....	42		
Punjab.....	20		

The varieties that had reactions intermediate between "immune" and "susceptible" to one or more races of *Melampsora lini* are listed in table 3. The seven differential varieties that were neither immune from nor susceptible to each race are included in this table. Two selections of Buda × (19×112) (C. I. 821 and 826) had reactions identical with those of the differential line of Buda. The reaction of Abyssinian (C. I. 511) was similar to that of the differential selection of Akmolinsk (C. I. 515-1). The other varieties listed in table 3 deviated from each of the differential varieties in their reaction to one or more physiologic races of rust. This suggests that these varieties possess factors or combinations of factors modifying rust reaction not possessed by any of the 11 differential varieties listed in table 1. It is probable that several of these varieties may be of value in differentiating new physiologic races of flax rust.

The reaction to North American races of rust of several varieties listed in table 3 was found to be affected by small variations in environment and thus was extremely variable. The reactions of "pale pink" (C. I. 649), Indian type 55, "pale blue" (C. I. 176), and "pale blue crimped" (C. I. 647) were especially subject to modification by a variable environment.

TABLE 3.—Reaction of varieties of seed flax (*Linum usitatissimum*) having intermediate differential reactions to physiologic races of *Melampsora lini*

Group and variety	C. I. No.	Predominant reaction ¹ of 30-day-old plants to physiologic race No. —															
		1	2	3	4	5	7	8	9	10	16	19	20	21	22	23	24
Petals broad, flat:																	
Abyssinian:																	
From Fergana.....	511	R	R	S	R	S	S	R	R	S	S	S	S	S	S	S	R
Abyssinian.....	701	I	I	R	I	R	R	S	I	I	S	S	S	S	S	S	I
American and European:																	
Blue-flowered:																	
Buda.....	270-1	R	S-	SR	S	R+	I	S	S-	R+	S	S	I	S	S	SR	S-
Williston Brown.....	803-1	S-	S-	S-	S	S-	R	S-	S-	R	S-	S	S	S	S	S-	S-
Pink-flowered:																	
Lethbridge Golden.....	23	S	S	S	S	S	R	R	S	R	S	S	S	S	S	SR	S
Pale pink.....	649	R	R	R	R	R	R	R	R	R	R	S-	S-	R	S	SR	S
Williston Golden.....	25-1	R	S	R+	R+	R-	R+	S-	R	R+	R+	S	S	S	S	S	S
Argentine:																	
Argentina (Minn. 25-323).....	705-1	SR	SR	SR	SR	SR	S-	SR	SR	SR	SR	S	S	SR	S	SR	SR
Bolley (37-5990).....	879	R	R	R	R	R	S-	R	R	R	R	S	S	R	S	R	R
Kenya.....	709-1	R+	R+	SR	R	R+	R+	SR	R	R+	R+	S	S	SR	S	R	R
Indian:																	
Howard and Khan (5):																	
var. <i>commune</i> , type 55.....		R	R	R	R	R	S-	S	S-	R	S	S	S	S	S	S	S
var. <i>campestris</i> , type 68.....		R	R	R	R	R	S-	S	S-	R	S	S	S	S	S	S	S
var. <i>sativum</i> , type 121.....		R	R	R	R	R	S-	S	S-	R	S	S	S	S	S	S	S
Russian:																	
Akmolinsk.....	515-1	R+	R+	S	R+	S	S	R	R+	R	S	S	S	S	S	S	R+
Pale blue.....	176	R	R	S	R	S	S	S	S-	R	S	S	S	S	S	S	S-
Hybrid:																	
Selection Buda×(19×112).....	821	R	S-	SR	S	R+	I	S	R+	R	S	S	I	S	S	SR	S-
Do.....	826	R	S-	SR	S	R+	I	S	S-	R+	S	S	I	S	S	SR	S-
Selection (19×112)×Buda.....	822	R	S-	SR	S	R+	I	S	S-	R+	S	S	I	S	S	SR	S-
Selection Buda×(19×112).....	827	R	S-	SR	S	R+	I	I	I	I	I	S	S	I	S	I	S-
Petals narrow, margins incurved or crimped:																	
Pale blue crimped ²	647	S-	S-	S-	S-	SR	S-	S-	S-	S-	S-	S	S	S-	S	S-	S-

¹ I=immune, R=resistant, SR=semiresistant, and S=susceptible. Plus and minus signs indicate somewhat more or less resistance or susceptibility than the letter designating the host reaction.

² Immune from physiologic race 11.

The varieties in which the rust reaction was so mixed that it was difficult to determine the predominant reaction with each race are listed in the following tabulation. Six of the 11 varieties listed in this table were pure for susceptibility to South American races 19, 20, and 22.

Varieties of seed flax (Linum usitatissimum) too heterogeneous to classify as to predominant type of reaction to physiologic races 1, 2, 3, 4, 5, 7, 8, 9, 10, 16, 19, 20, 21, 22, 23, and 24 of Melampsora lini in greenhouse tests

Group and variety	C. I. No.	Group and variety	C. I. No.
Mediterranean:		Hybrid:	
Crete.....	¹ 31-1	Bolley (37-5066).....	¹ 876
Cyprus.....	689	Long No. 125.....	¹ 356
Russian:		Reserve X Morye (19X112).....	486
Akmolinsk.....	² 520	Tammes, type 1 (common blue).....	765
North Caucasian.....	³ 620	Tammes, type 3.....	767
Winter:			
Roman Winter.....	¹ 470		

¹ Susceptible to races 19, 20, and 22.

² Susceptible to races 19, 20, 21, and 22.

³ Some plants immune from each race.

DISCUSSION

The production of rust-immune varieties of flax has been rendered more difficult by the discovery of new physiologic races of *Melampsora lini* to which all the varieties that had previously been considered immune were susceptible. Henry (3) noted that certain strains of Argentine flax, strains of Williston Golden, several varieties obtained from India, and Ottawa 770 B were immune in tests made in Minnesota. In his hybridization studies, he used selections of Argentine, Ottawa 770 B, and an Indian variety, Bombay, as supposedly rust-immune parents. In tests reported upon in this paper, all strains of Williston Golden were found to be highly susceptible to several races of flax rust prevalent in North America, while Bombay and several other Indian flaxes that were previously considered to be immune were susceptible to race 24 collected at Fargo, N. Dak. Ottawa 770 B and certain Argentine strains were immune from all races collected in North America but were susceptible to race 22 from South America. All of Henry's supposedly rust-immune hybrid strains that have been tested by the writer have been found to be susceptible to certain races and to have the reaction of the supposedly rust-immune parent. Myers (6) used Newland (C. I. 188) and a strain of Bolley Golden (C. I. 644), in addition to Ottawa 770 B and an Argentine selection, as immune parents in his studies on inheritance of rust reaction in flax. In the present studies all of these varieties were found to be susceptible to race 22 and also, with the exception of Ottawa 770 B, to races 19 and 20 from South America. Not one of the 201 varieties tested was immune from or resistant to all of the 24 races of flax rust thus far differentiated. These varieties had been selected for testing because of their diverse morphologic type, their agronomic importance, or their reported resistance to or immunity from rust. While all possibilities have not been exhausted there is a basis for doubt as to the existence of a variety of common flax immune from all races of rust.

There is a possibility, however, that a variety immune from all races of flax rust could be developed by hybridization. The varieties that apparently have the same factor for immunity as J. W. S. and

those that apparently have the same factor as Bombay were immune from races to which the Argentine type flaxes and Ottawa 770 B were susceptible. The latter varieties were, in turn, immune from those races of rust to which J. W. S. or Bombay were susceptible. No data exist as to combining in one variety these factors for immunity. Myers (6) found in a cross between two immune varieties, Newland and Ottawa 770 B, that immunity was conditioned by a single dominant factor in each variety and that the factors in the two varieties were not allelic. In the cross between the immune varieties Ottawa 770 B and Argentine selection (C. I. 438), his results suggested that C. I. 438 carried the same factor that conditioned immunity to Ottawa 770 B and also a factor for resistance that was allelic to the factor for immunity carried by Newland. The susceptibility of C. I. 438 to races 19 and 20, and the immunity of Ottawa 770 B from these races would indicate that Myers' suggestion of identical factors for immunity in these two varieties was not correct. The factor for immunity from the North American rusts used by Myers in C. I. 438 was probably allelic to the factor for immunity from North American rusts and races 19 and 20 from South America in Ottawa 770 B. This difference between the immune factors in these two varieties would not be apparent with the rusts used by Myers. If the factors for immunity carried by either Bombay or J. W. S. are not allelic to the factor for immunity carried by either Ottawa 770 B or Newland, it should be possible to develop a variety immune from all the known races of *Melampsora lini*.

There is a relatively wide range of flax varieties and types available for parental material having the resistance of the Argentine differential. Varieties in this group included American pink-flowered seed flaxes and Argentine, Indian, Mediterranean, Russian, and hybrid types. Choice of varieties available for parental material having the factors for immunity of the other three immune groups is relatively limited. Of the 20 varieties and selections having the immune reaction of Ottawa 770 B, 13 were Ottawa 770 B hybrids. Only 4 varieties had the immune reaction of J. W. S. Two of these were apparently identical strains of Tammes light-blue type 2. All 8 varieties that had the immune reaction of Bombay were Indian type flaxes or hybrids in which an Indian type flax was a parent.

Even if it is found possible to develop a strain of flax immune from all known races of *Melampsora lini*, the possibility of the rust hybridizing and developing a new race capable of attacking the immune flax should not be overlooked. In temperate regions, flax rust overwinters in the telial stage and the initiation of infection in the spring is dependent upon a natural hybridization process. It appears desirable to determine the inheritance of the factors governing different degrees of pathogenicity in the rust organism as well as those governing immunity in the host. Until this is done it seems desirable to exercise all possible precautions against the establishment in North America of the races attacking Ottawa 770 B, Newland, and Argentine flaxes. Similar precaution should be taken against the establishment in South America of races attacking Bombay and J. W. S.

SUMMARY

Ten new physiologic races of flax rust (*Melampsora lini*), in addition to the 14 previously reported, have been identified by the reaction of

11 varieties of flax. To differentiate the new races it was necessary to add 3 varieties, previously considered immune from rust, to the list of host testers. These are Argentine (C. I. 462), Bombay (C. I. 42), and Ottawa 770 B (C. I. 355).

All of the 201 varieties and strains of flax tested were susceptible to 1 or more of the 24 physiologic races of *Melampsora lini* that have been identified. These varieties and strains were selected for testing because of their diverse morphologic type, their commercial possibilities, or their reported resistance to or immunity from flax rust. Flaxes of Argentine type and Ottawa 770 B remained immune from all races of rust collected in North America but were susceptible to one or more of the races from South America.

Bombay and J. W. S. were immune from the races of rust obtained from South America but were susceptible to one or more of the North American races.

Pathogenicity tests indicate that each of the differential varieties Argentine, Bombay, J. W. S., and Ottawa 770 B possesses distinct factors governing immunity from specific races of flax rust. Of 201 varieties tested, 48 had the reaction of Argentine, 8 the reaction of Bombay, 4 the reaction of J. W. S., and 20 the reaction of Ottawa 770 B to 16 physiologic races of *Melampsora lini*.

The races of flax rust with a rather limited varietal range continued to predominate in the seed-flax-producing area of the Midwest despite the presence of a number of the races possessing a wider varietal range.

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THE INFLUENCE OF TEMPERATURE, MOISTURE, AND FOOD UPON THE DEVELOPMENT AND SURVIVAL OF THE SAW-TOOTHED GRAIN BEETLE¹

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INTRODUCTION

The saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (family Cucujidae), has been reported from many parts of the world as a pest of a wide variety of stored-food products. It is the grain insect most frequently sent in by Minnesota farmers for identification. Many observations in regard to its life history have been made and numerous short accounts of its distribution, habits, and biology are available in various reports. Prior to 1926 the brief biological notes of Chittenden (6)² were almost the only ones of a quantitative nature that had been published. In 1926 Back and Cotton (3) reported their observations made under ordinary room conditions with no attempt to control either temperature or moisture. The object of the present work was to obtain more definite information on the life history of this insect over a considerable range of controlled conditions in relation to temperature, food, and atmospheric moisture.

METHODS OF STUDYING DEVELOPMENT

Mass populations of saw-toothed grain beetle adults were reared on the moist, balanced food mixture recommended by Haydak (8) and held in a constant temperature chamber at 32° C. and 75 percent relative humidity. Under these conditions it was possible to obtain large numbers of the insect in a relatively short time. Several hundred beetles were collected and placed in an oviposition chamber, a pint fruit jar containing a small amount of the food mixture and several irregularly broken pieces of a large cork stopper. The cork had previously been softened and made pliable by moistening and autoclaving.

The eggs, which are normally laid in cracks and crevices of food material, are covered with an adhesive substance which causes them, when first deposited, to adhere to each other and to the food particles. Cork was found to provide a very suitable surface for the females to oviposit on and from which to retrieve the eggs. Following oviposition, the pieces of cork were removed and the adhering beetles shaken into the culture jar for future use. The eggs adhering to the cork were readily observed by means of a binocular microscope and were picked off with a fine moistened brush. They were then placed individually on the inner walls of 2-dram vials some distance above a small supply of food. Nearly half of the hatching experiments had

¹ Received for publication June 28, 1939. Paper No. 1723 of the Scientific Journal series, Minnesota Agricultural Experiment Station.

² Italic numbers in parentheses refer to Literature Cited, p. 614.

to be discarded because of injury to the eggs during handling. The larvae left the empty eggshells attached to the walls, where they could be seen easily, making it unnecessary to locate and disturb the minute, white, fragile larvae among the food particles.

The adult beetles are very excitable, and as handling of them was unavoidable before each oviposition period considerable time elapsed before the deposition of eggs actually occurred. Oviposition periods of 2, 4, and 6 hours were tried, but it was found necessary to extend the period to 10 hours. The beetles were placed in the oviposition chamber at midnight to facilitate the measuring of the development periods. Including the time required to retrieve them, the eggs were from 1 to 14 hours old before they were exposed to the various experimental conditions. Very few eggs were found to have been laid in the first 4 hours, however, so it is assumed that the majority of them were not over 6 to 7 hours old.

Eggs were placed singly in individual 2-dram vials which contained sufficient food and were stoppered with perforated corks covered with small pieces of silk bolting cloth. The vials containing the food had previously been conditioned at the desired temperature and humidity for at least 36 hours. Groups of 30 vials, each vial containing one egg with food, were placed in small desiccators held at constant temperature. Various known concentrations of sulfuric acid in water were used to control the atmospheric moisture within the desiccators. The acid concentrations required to give the desired atmospheric conditions were calculated from a graph taken from Wilson (16). The desiccators were at no time removed from the cabinets during the experimental period, and the vials were out only for a few minutes daily during the critical periods, preliminary tests indicating approximately when observations were to be made. It is believed that the atmosphere of the vials reached an equilibrium with the atmosphere in the desiccators within a reasonably short time since the desiccators were small and the vials were but a short distance above the acid solutions.

The various stages of the insect were exposed to temperatures of 15°, 20°, 25°, 30°, 35°, and 40° C. in cabinets controlled by toluene-mercury thermostats which did not vary more than $\pm 0.5^\circ$. At least three replications were made in each category.

Two levels of atmospheric moisture or saturation deficiency were at first chosen, 5 mm. and 12.5 mm. As the work progressed it was thought desirable to provide a wider range of moisture conditions and a third level, 22.5 mm. of saturation deficiency, was added.

Three rather different types of food commonly infested by the saw-toothed grain beetle were used, namely, rolled oats, English walnuts, and raisins. A sufficient quantity of each for the entire experiment was obtained at the beginning of the study. Each vial contained enough of the food material to more than support the insect, but not enough for the larva to burrow into and become covered. The larva, therefore, was more or less exposed to the atmosphere at all times. Raisins, because of their hygroscopic nature, did not lend themselves to some of the conditions of the experiment. The young larvae frequently became enmeshed in the sticky syrup produced by the raisins and in such cases there was a high mortality.

Two measures of moisture were utilized in this work, saturation deficit and relative humidity. The first is a measure of the evapora-

tive power of the atmosphere and, being uniform at different temperatures, may be applied to the study of the direct effect of atmospheric moisture upon insect growth. At a 5-mm. saturation deficiency, for example, the rate of evaporation is theoretically constant at the temperatures 35° 30°, 25°, and 20° C.

Atmospheric moisture, on the other hand, is absorbed by food materials in proportion to the relative humidity of the air. The relative humidities corresponding to a 5-mm. saturation deficiency at 35°, 30°, 25°, and 20° C. are 88, 84, 79, and 72 percent respectively. In preliminary tests rolled oats exposed in desiccators at these temperatures and humidities for 4 days and then dried at 105° for 24 hours showed a moisture content on a dry-weight basis of about 21.6, 22.8, 18.8, and 15.9 percent respectively. In the analyses of Atwater and Bryant (1) soft-shelled walnuts averaged 2.5 percent moisture. Finely divided soft-shelled walnuts exposed to relative humidities of 46.7 and 88.0 percent at 35° for 5 days and then dried over sulfuric acid for 10 days gave approximate moisture-content values of 1.7 and 6.6 percent. Walnuts appear to be less hygroscopic than rolled oats owing possibly, to their high oil content and to their reduced surface.

If moisture were held constant in terms of relative humidity, regardless of temperature, the food moisture content would be constant, but the rate of evaporation from the surface of the insect would then be variable because of the differences in saturation deficiency. By holding one value constant the other will be variable. It is realized, therefore, that in studying the effects of temperature alone, the moisture relationships are not entirely in agreement. Although the rates of evaporation are constant, the moisture content of the food material differs slightly at each temperature, and the changes in the rate of development cannot be said to be due entirely to the effects of temperature. The larvae in a condition of higher relative humidity, because of a higher percentage of water in the food, will be able to replace water loss with greater ease than those at a lower relative humidity. Much greater effects, in general, can be attributed to temperature, and it is believed that errors due to the variation in food moisture content are not very great. On walnuts, the moisture content of which is little affected by wide variations in humidity, the error is probably much reduced.

In these experiments the walnuts were divided to permit accessibility of the food to the newly hatched larvae. Schwardt (14) observed that finely divided foods of high oil content were detrimental to larval development. He stated that when the larvae crawl upon or through divided oily food they are brushed on all sides and become coated with a thin film of oil. If the food particles are larger the larvae can pass through the interstices and only touch the food with their tarsi and part of the ventral surface of the abdomen. In this study it was found that ground walnuts were fatal to the larvae whereas chopped walnuts had no detrimental effects.

In the statistical procedure the following formula of Fisher (7) was used:

$$S. E. = \frac{\sqrt{\frac{S(x^2) - S(x)\bar{x}}{N-1}}}{\sqrt{N}}$$

The formula for determining the error of the difference between two means is as follows:

$$\frac{(\bar{x}_1 - \bar{x}_2)}{\sqrt{S.E._1^2 + S.E._2^2}}$$

when \bar{x} is the mean, and S. E. is the standard error of the mean.

RESULTS OF DEVELOPMENT STUDIES

EGG

The eggs are usually laid in groups of 3 or 4 in the cork supplied for oviposition. Frequently, however, as many as 20 to 30 were found in a cluster.

From inspection of the data in table 1, it is seen that atmospheric moisture has little or no effect on the rate of development of the egg. The length of the egg stage varies considerably with the temperature. Eggs failed to develop at 15° or at 40° C. At 20° somewhat more than 12 days were required for them to hatch, while the greatest rate of development occurred at 35° when only a little over 4 and a fraction days were necessary. Although separate series of data are given for each of the three foods, all are computed on a similar basis and a comparison of the results will give an idea of the reliability of the differences caused by temperature changes.

TABLE 1.—The development of the saw-toothed grain beetle under various conditions of temperature, moisture, and food

Food and temperature (° C.)	Saturation deficit	Relative humidity	Egg		Larva			Pupa		Total		
			N	\bar{x}	N	\bar{x}	Standard error	N	\bar{x}	N	\bar{x}	Standard error
Rolled oats:	Millimeters	Percent	Days	Days	Days	Days	Days	Days	Days	Days	Days	Days
20°	5.0	71.6	19	12.79	19	39.74	0.66	18	16.33	18	69.06	0.90
	12.5	28.7	36	11.58	36	44.64	.85	14	17.43	14	73.64	1.02
	5.0	78.8	45	6.29	45	15.51	.27	45	8.53	45	30.31	.23
25°	12.5	47.3	32	6.75	32	17.66	.48	31	8.13	31	32.23	.43
	22.5	5.2	44	6.84	44	23.61	.37	40	8.55	40	39.15	.43
	5.0	84.3	36	4.89	36	10.28	.19	36	5.50	36	20.67	.17
✓30°	12.5	60.7	36	4.99	36	12.28	.31	36	5.61	35	22.80	.30
	22.5	29.2	36	5.00	36	15.72	.25	34	5.74	34	26.41	.27
	5.0	88.1	43	4.16	43	9.37	.14	43	4.47	43	18.00	.14
35°	12.5	70.4	33	4.30	33	10.85	.45	33	4.67	33	19.82	.44
	22.5	46.7	45	4.31	45	14.84	.25	45	4.71	45	23.87	.30
English walnuts:												
20°	5.0	71.6	31	12.61	31	44.26	.74	17	15.12	17	70.56	1.00
	12.5	28.7	10	12.32	19	52.47	.86	3	16.00	3	77.67	1.78
	5.0	78.8	44	6.52	44	18.32	.29	40	7.83	40	32.73	.33
25°	12.5	47.3	31	6.65	31	21.74	.52	25	8.32	25	36.52	.66
	22.5	5.2	50	6.70	50	33.08	.77	31	9.29	31	48.55	1.25
	5.0	84.3	29	5.03	29	13.59	.36	28	5.68	28	24.11	.43
✓30°	12.5	60.7	31	5.03	31	15.74	.31	28	5.93	28	26.54	.37
	22.5	29.2	50	4.98	50	21.20	.46	30	5.47	30	30.97	.52
	5.0	88.1	37	4.00	37	15.30	.29	22	4.45	22	23.68	.45
35°	12.5	70.4	32	4.09	32	16.09	.47	14	4.79	14	25.14	.71
	22.5	46.7	29	4.24	29	21.76	.96	14	4.67	14	29.57	.89
Raisins:												
20°	5.0	71.6	3	12.00	3	51.00	-----	3	17.67	3	80.67	-----
	5.0	78.8	10	6.20	10	30.10	-----	5	8.20	5	40.80	-----
25°	12.5	47.3	10	6.20	10	32.50	-----	10	8.40	10	47.20	-----
	5.0	84.3	4	4.00	4	21.50	-----	1	6.00	1	36.00	-----
30°	12.5	60.7	8	4.00	8	27.50	-----	8	5.63	8	36.63	-----

LARVA

Since the eggs failed to hatch at 15° C., some were hatched at 20°, the next higher point in the temperature series, and the newly hatched

larvae were placed on rolled oats at a saturation deficiency of 5 mm. and a temperature of 15°. The larvae failed to develop. Under the same food and atmospheric moisture conditions at 20°, larvae developed in an average of 39.74 days as compared with 15.51 days at 25°. On rolled oats the optimum temperature for development is about 35° where 9.37 days are required for the larval stage, while at 30° the developmental time is 10.28 days. With walnuts as food, and under the same moisture conditions, the developmental optimum appears nearer to 30°, where 13.59 days are required as compared with 15.30 days at 35° C.

In general the data show significant differences in the rate of larval development at the three moisture levels within each temperature. Larval development tends to be more rapid at a higher humidity. The differences in developmental time between the moisture levels are greatest at 20° C. on both rolled oats and walnuts, because of the longer periods involved; in other words, differences in the developmental period decrease with an increase in temperature. Between 12.5 mm. and 22.5 mm. of saturation deficiency the data differ more because of the greater spread in atmospheric moisture conditions. Because of insufficient numbers, the raisin data are not statistically treated. From inspection, however, they appear to show the same trend as the other foods.

On the basis of the rate of development, rolled oats is superior to either walnuts or raisins as food for the saw-toothed grain beetle. The raisin data are admittedly lacking in sufficient replications and are presented only as an indication of the value of raisins as food for this insect. Comparing the rates of development at 25° C. and 5-mm. saturation deficiency, the larvae completed development in 15.51 days on rolled oats, 18.32 days on walnuts, and 30.1 days on raisins.

PUPA

The rate of development of the pupa varies with the temperature, the maximum being 16 to 17 days at 20° C. and the minimum about 4.5 days at 35°. From inspection of the data, the atmospheric moisture or type of food shows little or no effect on the rate of development, although here, as in the egg stage, any actual differences would be slight and would be obscured by the short developmental period and the relatively long intervals between observations. The data, however, are mostly rather uniform within each temperature regardless of humidity, and the values in each class on the different foods approach each other.

TOTAL DEVELOPMENTAL PERIOD

Since food and atmospheric moisture conditions have little or no measurable effect on the rate of development of the egg and pupa, the rate of development under the various conditions for the entire period from egg to adult is similar to that of the larva. On rolled oats at a 5-mm. saturation deficiency, the insect completes its development in the shortest time of 18.0 days at 35° C., while the longest mean period occurs at 20° where 69.06 days are required. With walnuts as food the data for total development at 30° are not significantly different from those at 35°. The optimum condition lies somewhere between these temperatures. The shortest mean period on walnuts at a

5-mm. saturation deficiency is 23.68 days at 35°, while the maximum, which occurs at 20°, is 70.59 days. On raisins the developmental periods appear from the few available data to be somewhat longer. At 30° and at the high humidity, one complete life cycle requires about 36.0 days, while at 20° the complete developmental time is 80.67 days.

The longest total period of individual development recorded in these experiments was 84 days on raisins at 20° C. and a 5-mm. saturation deficit. Under the same conditions, but on rolled oats instead of raisins, the longest period was 78 days, the shortest 64 days. The comparable data for 35° with rolled oats as the food are 20 and 17 days, respectively.

DISCUSSION

Chapman and Baird (5) determined the duration of the developmental stages of *Tribolium confusum* Duval at several temperatures

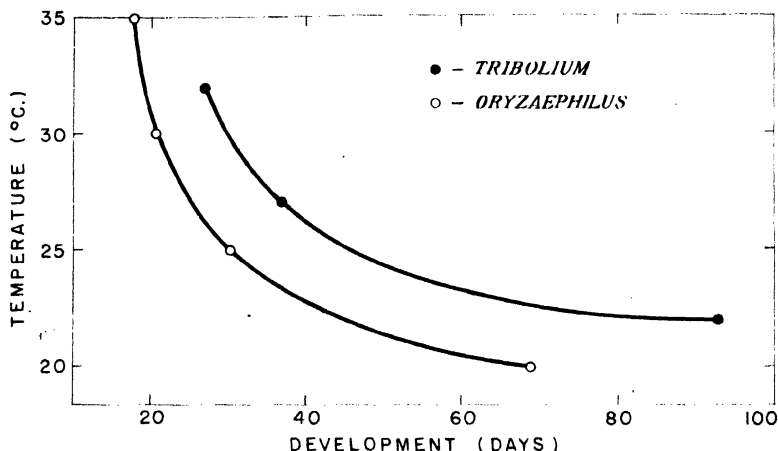


FIGURE 1.—Comparison of the total developmental periods at different temperatures for *Oryzaephilus surinamensis* fed on rolled oats at 5 mm. saturation deficiency and *Tribolium confusum* fed on whole-wheat flour at 75 percent relative humidity.

and 75 percent relative humidity. In figure 1 are shown these data in comparison with the present determinations for *Oryzaephilus*. The data, although not exactly comparable, are nearly so. In the second species the data plotted are those at 5 mm. saturation deficiency, but the latter is close enough to 75 percent relative humidity that not over 1½ days correction would be necessary for developmental periods of 70 days or more. Furthermore, *Oryzaephilus* was reared on rolled oats whereas *Tribolium* had whole-wheat flour as food. These foods, however, are not widely different. The comparison of the two species with respect to the time required for development from egg to adult shows *Oryzaephilus* to be considerably faster than *Tribolium confusum* in its development. At 30° C. the difference amounts to about 8 days; at 22°, to about 52 days. Therefore the development of *Tribolium confusum* under somewhat comparable conditions may take over twice the time required by *Oryzaephilus*.

Holdaway (9) studied the development of *Tribolium confusum* at several relative humidities and a single temperature (27° C.) in connection with population studies. He found a 24.4-percent increase in the total developmental period when the relative humidity was reduced from 75 to 25 percent. In the present studies with *Oryzaephilus* at 25° a comparable lengthening of the period was found amounting to a 25.9-percent increase when the saturation deficit was increased from 5.0 to 22.5 mm. pressure (a drop from 78.8 to 5.2 percent relative humidity).

The fact that stored-product insects are able to develop in very dry food is well known. Certain insects can gain water from an atmosphere which is nearly saturated, the water entering through channels other than the mouth. Babcock (2) pointed out that through oxidation of food material a considerable quantity of water is produced which many animals use to carry away nitrogenous wastes in solution. Insects living on dry foodstuffs, however, excrete solid uric acid and are able to retain the water of metabolism which would otherwise be used in excretion.

The water content of their food has been shown to influence the proportion of water in insects. Insects that feed on food of high moisture content contain a much higher percentage of water than those that live on dry materials. Living on dry food, the insects themselves contain a higher percentage of water than there is in the food. Schultz (13) has shown that larvae of *Tenebrio molitor* L. fed on bran pass feces of which 80 percent is undigested food but lower in moisture than the original bran. Buxton (4) explained this as the result of wasteful eating as well as the formation of water of metabolism. When food is eaten wastefully the small amount of free water contained in it is probably extracted by mechanisms in the rectum, which results in dry excreta. Schwarzt (14) observed the saw-toothed grain beetle develop in food containing practically no free water.

According to Buxton (4) starved larvae of *Tenebrio molitor* lose weight when exposed to a humidity of 80 percent or lower, but if kept at 90 percent and temperatures of 23° or 30° C. they gain weight. He stated that the phenomenon is not similar to the hygroscopy of hair and other dead materials, but that it is due to biological activity. Water from the liquid in the tracheoles is thought to be continuously secreted into the insect body, thereby increasing the concentration of the tracheole liquid and causing atmospheric moisture to be absorbed by it.

Stored-product insects are able to conserve moisture in various ways, thus protecting themselves against desiccation. Mellanby (10) stated that practically all the water that evaporates from an insect is lost through the spiracles. When the spiracles are closed the air within the tracheae becomes saturated and in most cases remains so when the spiracles are opened. Otherwise, when the air becomes very dry, the air of the tracheae becomes dry, the tissues being unable to supply water fast enough. One of the best methods that insects possess for protecting themselves against loss of water, then, is their ability to close the spiracles. Certain factors, however, such as an increase in temperature, may accelerate metabolic activity and increase the rate of respiration, causing the spiracles to be opened more often and increasing the rate of water loss.

The effect of atmospheric moisture on a stored-product insect,

therefore, is in proportion to the rate at which moisture is being withdrawn from the insect and to the ease with which that moisture can be replaced. The longer time required for development on walnuts than on oats is probably due in considerable part to the lower hygroscopicity of the food.

LETHAL EFFECTS OF HIGH AND LOW TEMPERATURES

The lethal effects of high and low temperatures on insects are known to vary with the species, the developmental stage, and the other environmental factors, such as moisture. Data have been presented in the preceding paragraphs showing that the egg and larva of the saw-toothed grain beetle are incapable of developing at 40° and 15° C. To obtain further information on the reactions of this insect to lethal temperatures, its longevity was determined near both the upper and the lower limits of development. Only healthy adult insects, of which the sex and age were disregarded, were used in these experiments, since it was not feasible at the time to add greatly to the experimental work by determining the effects on all stages.

Oosthuizen (12), working with all stages of the confused flour beetle, *Tribolium confusum*, found some variation in the heat resistance of different stages at different temperatures. He showed that at 44° C. 50 percent of the adult beetles were killed in 7.4 hours and that changes in the atmospheric moisture conditions had no effect at this temperature. At 46° only 64 minutes were required to kill 50 percent of the adults, but at this temperature moisture conditions affected the results, the beetles dying much sooner at 100 percent relative humidity and somewhat sooner at 0 and 30 percent than at 75 percent.

Temperatures between 44° and 46° C. are critical, and very slight changes in the intensity of heat between these points produce profound effects. The writers operated the same apparatus used by Oosthuizen, but it is felt that somewhat greater accuracy in measurement and control of the bath temperatures was attained. The temperatures recorded by Oosthuizen as 44° and 46° are actually slightly lower, although certainly not over 0.7° lower, and probably about half that much.

Adult beetles were exposed in a water bath arrangement and with a technique essentially the same as that described by Oosthuizen. Cages made of a fine-mesh wire screen were used instead of glass and bolting cloth. Thermometers were not placed in the cages as it was found that the desired temperature could be obtained there by maintaining the outside water bath temperature 0.7° C. higher. This difference was due to loss of heat from the exposed portions of each glass exposure chamber at the water surface. Temperatures were measured with a sensitive thermometer graduated in tenths of a degree centigrade. Cage temperatures did not vary more than $\pm 0.1^\circ$. The exposure chambers were conditioned for at least 4 hours prior to each experiment. As active saw-toothed grain beetles cannot live long without food, a small amount of food was provided in each cage. Exposed beetles were allowed 2 days for recovery before the percentage of mortality was determined. Each point plotted in figure 2 represents in excess of 150 individuals.

Tattersfield and Morris (15) have shown that mortality data can best be compared at the point at which 50 percent of the individuals

survive. The writers' data for adults of *Tribolium confusum* show somewhat lower values than those of Oosthuizen. At 44° C. and 30 percent relative humidity, 50 percent of the beetles were killed in a little over 4 hours while at 46° and 50 percent relative humidity about 40 minutes were required, as compared with 7.4 hours and 60 to 75 minutes respectively as found by Oosthuizen. These differences show how important a fraction of a degree may be in shifting the time required to kill a group of insects.

The exposure periods necessary to kill 50 percent of the adults of the saw-toothed grain beetle decrease rapidly with slight increases in heat intensity between the temperatures tested. At 42° C. 50-percent mortality occurs in about 34 hours while at 44°, only 2° higher,

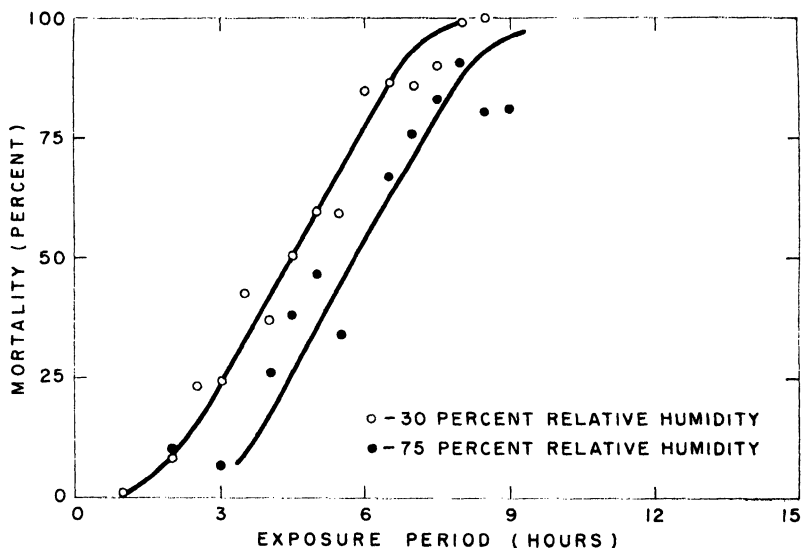


FIGURE 2.—Mortality of the saw-toothed grain beetle exposed for different lengths of time at 44° C. and relative humidities of 30 and 75 percent.

50 percent of the beetles are killed in approximately 4.5 hours at 30 percent relative humidity, and 5.7 hours at 75 percent relative humidity (fig. 2).

In general the saw-toothed grain beetle and the confused flour beetle are about equally resistant to high temperature. The chief difference between these species appears to lie in the higher sensitivity of *Oryzaephilus* to desiccation. At 44° C. this species is definitely more easily killed at 30 percent relative humidity than at 75 percent. At the same temperature and humidities, *Tribolium confusum* is unaffected by the moisture differences. It appears likely that this fact is correlated with the greater susceptibility of *Oryzaephilus* to desiccation at ordinary temperatures.

Nagel and Shepard (11) studied the effects of low temperatures on all stages of *Tribolium confusum*. The exposure periods necessary to obtain a 50-percent mortality of the adults at 7°, -6°, -12°, and -18° C. were 336, 8.4, 0.23, and 0.15 hours respectively.

Adult saw-toothed grain beetles were exposed in small fine-mesh wire screen cages, each cage containing approximately 50 beetles and

a small quantity of food material. The cages were placed in dry desiccators in refrigerated cabinets at the desired temperatures. No attempt was made to control the atmospheric moisture conditions, but the temperatures were maintained to within $\pm 1.0^{\circ}\text{C}$. Two days were allowed for the insects to recover before mortality was determined. Exposures were made at two temperatures, 10° and 2° . The exposure periods to obtain 50-percent mortality were 30 days and 105 hours respectively. It is impossible from these determinations to say whether *Oryzaephilus* differs significantly from *T. confusum* in resistance to low temperature.

SUMMARY

A study was made of the development of all stages of the saw-toothed grain beetle (*Oryzaephilus surinamensis* L.) under various conditions of temperature, atmospheric moisture, and food. Rearings were made or attempted at temperatures of 15° , 20° , 25° , 30° , 35° , and 40°C ., at saturation deficiencies of 5, 12.5, and 22.5 mm., and with rolled oats, English walnuts, and raisins as food.

At 5 mm. saturation deficit and on rolled oats as food, the total life cycle from egg to adult requires 69.06 days at 20°C ., 30.31 days at 25° , 20.67 days at 30° , and 18 days at 35° . With rolled oats as food, the developmental optimum appears at 35°C . while with walnuts, the developmental optimum occurs between 30° and 35° . Eggs larvae failed to develop at 15° and 40° . In general, development is more rapid at the higher humidities. The egg and pupal stages appear to be little affected by atmospheric moisture conditions. On the basis of the rate of development, rolled oats are superior to either walnuts or raisins as a food for the saw-toothed grain beetle.

Adults of the saw-toothed grain beetle and the confused flour beetle were exposed to high temperatures. At 44°C . and 30-percent relative humidity, 50 percent of the flour beetles were killed in a little over 4 hours while at 46° and 50-percent relative humidity about 40 minutes were required. At 42° , 50 percent of the adult saw-toothed grain beetles were killed in 34 hours while at 44° the exposure periods were approximately 4.5 and 5.7 hours at 30- and 75-percent relative humidities respectively.

Adult saw-toothed grain beetles exposed at 10° and 2°C . gave 50-percent mortality values at 30 days and 105 hours respectively.

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RETENTION BY SOILS OF THE NITROGEN OF VARIOUS COMPOUNDS AS SHOWN BY SUBSEQUENT PLANT RESPONSE¹

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INTRODUCTION

Although nitrates and nitrites dissolved in the soil solution are not held by the solid phase of the soil against leaching, the ammonia form of nitrogen is strongly retained. Soil chemists have known these facts for many decades. The reactions of many other nitrogenous compounds with soils, however, have been but little studied. Do soil solids generally fail to retain the nitrogen contained in all of the more or less acidic anions like nitrate and nitrite? On the other hand, is the nitrogen in all the cations which exhibit only moderately basic properties retained by the soil solids as is that in ammonium ions? Experiments to test these questions further were undertaken in this study. Regardless of how accurately the reactions of the soil with moderately acidic or basic ions may be foretold, questions regarding the retention of nitrogen-carrying units of intermediate or amphoteric properties are not answered thereby; nitrogenous compounds with neither pronounced anionic nor cationic properties were accordingly included in this study.

With but few exceptions, soil-fertility investigations directed toward ultimate application to field conditions must encounter the factor of percolating waters resulting from natural rainfall or irrigation. Soluble nitrogen-containing units, whether in ionic form or not, may be dissolved in the irrigation water and applied to the soil in this way. They may also arise in the soil either through the solution of nitrogenous fertilizers previously applied in a dry state, or as the end products of purely chemical reactions or of biological activity. In this connection, the soluble intermediate products of such transformations cannot be neglected. With such an important element of fertility as nitrogen, knowledge of the reactions with the soil of the various units carrying this element is important for efficient progress on many problems.

METHODS

The method described by Conrad and Adams (3)² was used because it not only gives a semiquantitative estimate of the reaction of the compound in question with the soil, but also indicates the effect of the compound on the growth of the test plants. In figure 1 is shown a column of pots illustrating the general method used. Each 4-inch pot, previously coated with asphaltum paint, was provided with a square of waxed paper to cover the drainage hole. Usually 400 gm.

¹ Received for publication September 5, 1939. Investigations of the Division of Agronomy, California Agricultural Experiment Station, Davis, Calif.

² Italic numbers in parentheses refer to Literature Cited, p. 630.

of dry soil deficient in nitrogen was then added to each pot. The three pots of each column were stacked with the bottom one nesting in the drainage can and with the consecutive pots in the column held apart by a cannery-tin top punched with a large hole. The large 5-inch pot above, equipped with a single-holed rubber stopper, was used as a reservoir for the test solution. A piece of glass tubing drawn out to a long taper and inserted in the hole of the rubber stopper constituted, with the stopper, a needle valve. The rate of flow was then easily regulated by raising or lowering this tapered tubing. Thus the test solution was allowed to drip slowly down upon the column of pots. At the outset, sufficient solution was added to the reservoir to wet the soil completely and to give some drainage through the bottom pot. A somewhat greater excess was used when

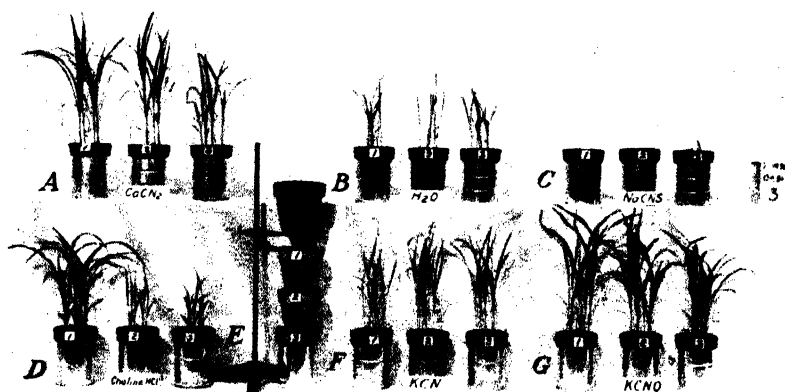


FIGURE 1.—Effect on plants of the retention of nitrogen from various percolating solutions. At *E* the solution being tested slowly dripped from the reservoir above, down upon the column of 4-inch pots (1, 2, and 3), each containing about 400 gm. of dry soil deficient in nitrogen. After percolation the pots in the columns were taken down and subsequently cropped. The test solution used in each case is shown on the card leaning against pot 2 of that column. The soil showed practically no ability to retain nitrogen in the anionic units of KCN (*F*), KCNO (*G*), and NaCNS (*C*). The nitrogen of choline hydrochloride (*D*) was completely retained in the top pot. CaCN_2 (*A*) showed intermediate properties.

these leachings were to be analyzed. In general the solutions were percolated through the columns as rapidly as possible. Usually within 12 hours after percolation started, the solution had disappeared from the reservoir above and from the surface of the soil in the top pots, and water had appeared in the drainage can. Except as noted below, no effort was made to prolong the actual percolation. Generally in from 24 to 48 hours after percolation the pots were taken down and cropped, each pot being provided with a separate drainage can. In some tests, the seed had been planted in the dry soil before percolation; in others, planting was deferred until a few days or more after the columns were dismantled. Double Dwarf milo and Double Dwarf darlo, varieties of grain sorghum (*Sorghum vulgare* Pers.) commercially grown in California, were used as test plants.

In experiments reported with phosphorus compounds (2), the pots

were weighed before and after percolation to evaluate the uniformity in the amounts of moisture held by the soil in the various pots of the column. These showed variations of less than 10 percent of the moisture present between that held by the top pots and that held by the bottom ones. Even though weighings of pots were only occasionally made for the tests reported herein, the variations in moisture held by the soil were assumed to be no larger than in earlier studies.

Nonretention of the compound or at least its nitrogen-containing units by the solid phase of the soil was indicated by the nearly equal distribution of the nutrient (or toxic) effects of the solute, assuming equal distribution of the solvent among the pots of the column. Unequally enhanced (or in cases of toxicity, injured) growth among the pots of the column, with the divergence from the corresponding pots of the distilled-water columns being greater in the top pot and successively less or absent in lower pots, indicated retention. Nitrogen-containing (or in cases of injury, toxic) units that were retained had reacted either physically or chemically with the soil by becoming more or less firmly affixed to the solid particles of the soil and had thereby been more or less completely removed from the solvent as it trickled by.

The soils used in this study were as follows:

Yolo fine sand (a loamy fine sand), C-11, was secured as a deep subsoil sample from a Yolo soil on the southern bank of Putah Creek on the university farm at Davis. This soil and the next two are described in the Soil Survey report of the Dixon area (4).

Yolo silt loam, C-62, was secured as a surface sample from the experimental plots of the Division of Agronomy, at Davis; it had previously been cropped to small grains.

Yolo fine sandy loam, C-68, was secured as a subsoil sample from a depth of 1 to 2 feet from the agronomy experimental area on land that had been planted to a variety of crops and that had lain fallow for a few months before the sample was collected.

Fresno fine sandy loam, C-10, was secured as a surface-soil sample from a vineyard about 10 miles northwest of the city of Fresno. This soil is described in the soil survey of the Middle San Joaquin Valley (9).

The nitrogen compounds used in these experiments were as follows:

C. p. chemicals— $\text{Ca}(\text{NO}_3)_2$, NaNO_3 , NaNO_2 , KCN , KCNO , picric acid, NaCNS , $\text{K}_4\text{Fe}(\text{CN})_6$, $\text{K}_3\text{Fe}(\text{CN})_6$, $\text{NH}_4\text{OOCCH}_3$, $(\text{NH}_4)_2\text{CO}_3$, NH_4OH , $(\text{NH}_4)_2\text{HPO}_4$, $(\text{NH}_4)_2\text{SO}_4$, urea, hydrazine sulfate, and hydroxylamine sulfate.

Chemicals of other grades—acetamide (purified crystals), peptone (bacteriological), egg albumin (impalpable powder, soluble), gelatin (granular edible), blood albumin (technical), commercial sulfamic acid, and ammonium sulfamate; cyanamide (CaCN_2) bought as a fertilizer.

Chemicals of highest purity—guanidine carbonate, theobromine, glycine, hippuric acid, glutamic acid, formamide, diacyandiamide, phenylurea, thiourea, cyanuric acid, acetonitrile, and acetoxime, and hydrochlorides of methylamine, dimethylamine, trimethylamine, choline, semicarbazide, cysteine, and arginine.

Chemical of practical grade—tetramethylammonium chloride.

GROWTH STUDIES

The tests with the various nitrogen compounds were conducted as time and the supply of materials permitted. Included in each growth

series were columns percolated with distilled water to serve as checks in evaluating the data secured with the various solutions. The main interest in these data arises from the behavior of different forms of nitrogen on the basis of the chemical properties of each. Since the various growth series could not be arranged according to the chemical properties of the compounds, it seemed best to report the data in table 1 not as the compounds were grouped in the separate growth series but in a recapitulated form arranged as follows: Section A, nitrogen in anionic units; B, nitrogen in cationic units; C, nitrogen in amphoteric or approximately neutral units; and D, checks with distilled water and cultural data applicable to each growth series.

In section A were placed sulfamic and picric acids, both fairly strong acids, as well as the salts of either weak or strong acids. Nitro-urea, though somewhat stronger than acetic acid (13), is placed in section C for comparison with urea. All fairly strong bases containing nitrogen, as well as the salts of such bases, are in section B. Section C contains the weaker acids and weaker bases, as well as the amphoteric and approximately neutral organic compounds. Cysteine, even though used as the hydrochloride, belongs to this group.

In sections A, B, and C of table 1 are columns headed "Growth series." By referring to the corresponding growth series in section D, the yields resulting from each solution can be compared

TABLE 1.—Retention of the nitrogen from various percolating solutions by the solids of different nitrogen-deficient soils in columns of three 4-inch pots as shown by the subsequent average yield of replicated cultures of Double Dwarf milo and darlo

A. NITROGEN IN ANIONIC UNITS

Percolating solution	Growth series (see sec. D below)	Repl-ications	Average green yield per pot			
			Pot 1, top	Pot 2, middle	Pot 3, bottom	Control
			Grams	Grams	Grams	Grams
Calcium nitrate, $\text{Ca}(\text{NO}_3)_2$	1a	3	18.6	14.4	14.8	14.8
Sodium nitrate, NaNO_3	1	3	14.7	24.4	21.7	19.2
Sodium nitrite, NaNO_2	1	3	14.3	18.5	19.7	15.4
Potassium cyanide, KCN	4	3	2.7	14.0	14.3	14.3
Potassium cyanate, KCNO	5	3	10.3	10.1	8.6	10.1
Sulfamic acid, HSO_3NH_2	3	3	1.0	1.0	1.1	1.0
Picric acid, $(\text{NO}_2)_3\text{C}_6\text{H}_2\text{OH}$	4	3	1.1	1.1	1.3	1.0
Sodium thiocyanate, NaSCN	4	3	3.2	1.1	1.1	1.1
Potassium ferrocyanide, $\text{K}_4\text{Fe}(\text{CN})_6$	4	3	1.2	1.3	1.4	1.0
Potassium ferricyanide, $\text{K}_3\text{Fe}(\text{CN})_6$	4	3	1.4	1.9	1.0	1.0
Calcium cyanamide, CaCN_2	2	3	8.2	5.3	1.9	1.9

B. NITROGEN IN CATIONIC UNITS

Ammonium acetate, $\text{NH}_4\text{OOCCH}_3$	3	3	10.3	1.9	1.7	15.0
Ammonium carbonate, $(\text{NH}_4)_2\text{CO}_3$	3	3	10.4	1.0	1.0	15.4
Ammonium hydroxide, NH_4OH	1	3	43.4	2.0	1.7	20.3
Ammonium phosphate, $(\text{NH}_4)_2\text{HPO}_4$	3	3	14.0	1.2	1.2	9.6
Ammonium sulfamate, $\text{NH}_4\text{SO}_3\text{NH}_2$	3	3	4.4	4.5	1.3	2.4
Ammonium sulfate, $(\text{NH}_4)_2\text{SO}_4$	1	3	48.7	1.5	1.2	26.6
Hydrazine sulfate, $\text{N}_2\text{H}_4\text{H}_2\text{SO}_4$	4	3	3.1	1.4	1.7	2.5
Hydroxylamine sulfate, $(\text{NH}_2\text{OH})_2\text{H}_2\text{SO}_4$	7	5	7.7	1.9	2.3	3.2
Tetramethylammonium chloride, $(\text{CH}_3)_4\text{NCl}$	7	3	19.6	1.7	1.8	13.7
Hydrochlorides:						
Trimethylamine, $(\text{CH}_3)_3\text{N.HCl}$	7	3	21.6	1.9	1.8	12.0
Dimethylamine, $(\text{CH}_3)_2\text{NH.HCl}$	7	3	21.7	1.6	1.6	13.0
Methylamine, $\text{CH}_3\text{NH}_2\text{HCl}$	7	3	24.0	1.4	1.5	11.1
Choline, $\text{HO}(\text{CH}_2)_2\text{N}(\text{CH}_3)_2\text{OH.HCl}$	5	3	22.7	1.6	1.4	11.1
Arginine, $\text{C}_6\text{H}_{14}\text{N}_4\text{O}_4\text{HCl}$	7	6	15.8	1.9	1.9	18.1
Semicarbazide, $\text{N}_2\text{H}_4\text{CONH}_2\text{HCl}$	4	3	7.2	2.5	2.5	14.1
Guanidine carbonate, $\text{NH}_2\text{C}(\text{NH}_2)_2\text{H}_2\text{CO}_3$	6	3	1.0	5.1	4.4	3.1

TABLE 1.—Retention of the nitrogen from various percolating solutions by the solids of different nitrogen-deficient soils in columns of three 4-inch pots as shown by the subsequent average yield of replicated cultures of Double Dwarf milo and darlo—Continued

C, NITROGEN IN AMPHOTERIC OR APPROXIMATELY NEUTRAL UNITS

Percolating solution	Growth series (see sec. D below)	Repl-ications	Average green yield per pot			
			Pot 1, top	Pot 2, middle	Pot 3, bottom	Control
			Number	Grams	Grams	Grams
Clycine, $\text{NH}_2\text{CH}_2\text{COOH}$	3	3	¹ 7.4	¹ 8.3	¹ 3.5	¹ 4.1
Do	7	5	¹ 15.8	¹ 9.2	¹ 2.1	¹ 10.2
Hippuric acid, $\text{C}_6\text{H}_5\text{CONHCH}_2\text{COOH}$	6	3	¹ 7.4	¹ 5.8	¹ 4.9	¹ 5.0
Glutamic acid, $\text{C}_5\text{H}_9\text{NO}_4$	4	3	¹ 17.8	¹ 8.6	¹ 8.0	¹ 3.0
Cysteine hydrochloride, $\text{HOOCCHNH}_2\text{CH}_2\text{SH.HCl}$	7	5	¹ 14.7	¹ 4.4	¹ 6	¹ 10.5
Formamide, HCONH_2	3	3	¹ 11.4	¹ 5.6	¹ 6	¹ 3.8
Acetamide, CH_3CONH_2	3	3	¹ 5.1	¹ 5.6	¹ 6.0	¹ 7.0
Dicyandiamide, $\text{NH}_2\text{C}(\text{NH})\text{NHCN}$	5	3	¹ 1	¹ 0	¹ 2	¹ 6.8
Urea, NH_2CONH_2	3	3	¹ 6.9	¹ 5.7	¹ 3.1	¹ 6.8
Do	1a	3	¹ 5.5	¹ 3.1	¹ 8	¹ 5.0
Phenylurea, $\text{C}_6\text{H}_5\text{NHCONH}_2$	3	3	¹ 2.2	¹ 3.7	¹ 3.2	¹ 5.8
Nitrourea, $\text{NH}_2\text{CONHNO}_2$	4	3	¹ 6.1	¹ 4.0	¹ 4.1	¹ 5.8
Thiourea, NH_2CSNH_2	6	3	¹ 4.04	¹ 0.4	¹ 2	¹ 4
Cyanuric acid, $\text{H}_3\text{C}_3\text{N}_3\text{O}_3$	3	3	¹ 4	¹ 4	¹ 4	¹ 7
Acetonitrile, CH_3CN	7	5	¹ 3.2	¹ 4.2	¹ 5.2	¹ 5.0
Acetoxime, $(\text{CH}_3)_2\text{C}=\text{NOH}$	7	4	¹ 2.9	¹ 2.5	¹ 2.7	¹ 3.1
Peptone	4	3	¹ 13.3	¹ 3.7	¹ 2.4	¹ 5.0
Egg albumin	4	3	¹ 9.8	¹ 7.1	¹ 2.4	¹ 4.7
Blood albumin	4	3	¹ 5.9	¹ 3	¹ 7	¹ 2.4
Gelatin	1	3	¹ 36.0	¹ 6	¹ 4	¹ 14.0
Theobromine, $\text{C}_7\text{H}_5\text{N}_3\text{O}_2$	7	5	¹ 10.0	¹ 8	¹ 8	¹ 2.9

D, CHECKS WITH DISTILLED WATER

Growth Series No.	Experiment began	Experiment ended	Repl-ications	Soil No.	Crop	Average green yield per pot			
						Pot 1, top	Pot 2, middle	Pot 3, bottom	Control
			Number			Grams	Grams	Grams	Grams
1	Mar. 19, 1938	Apr. 20, 1938	3	C-11	Milo	1.6	1.6	1.7	1.7
1a	Apr. 10, 1938	May 25, 1938	3	C-11	do				
2	Oct. 11, 1937	Nov. 14, 1938	3	C-62	do	1.0	.9	.9	1.4
3	Nov. 9, 1938	Dec. 16, 1938	3	C-68	Darlo	1.4	1.4	1.6	1.4
4	Sept. 17, 1938	Nov. 9, 1938	3	C-68	do	1.4	1.2	1.4	1.7
5	Sept. 28, 1938	Nov. 16, 1938	1	C-68	do	.6	.4	1.1	
6	July 16, 1938	Aug. 15, 1938	3	C-10	do	5.0	3.3	4.4	4.5
7	Mar. 20, 1939	Apr. 17, 1939	5	C-68	Milo	2.1	1.8	1.8	1.5

¹ Statistically different (5, p. 114) from the corresponding pots of the distilled-water columns as reported in sec. D. $P=0.01$ or less.

² Statistically different (5, p. 112) from the value in the column to the right and for the pot next below it in the column of pots. P lies between 0.02 and 0.05.

³ Statistically different (5, p. 114) from the corresponding pots of the distilled-water columns as reported in sec. D. P lies between 0.01 and 0.02.

⁴ Statistically different (5, p. 114) from the corresponding pots of the distilled-water columns as reported in sec. D. P lies between 0.02 and 0.05.

⁵ Statistically different (5, p. 112) from the value in the column to the right and for the pot next below it in the column of pots. $P=0.01$ or less.

⁶ Statistically different (5, p. 112) from the value in the column to the right and for the pot next below it in the column of pots. P lies between 0.01 and 0.02.

with the yields similarly and simultaneously secured by using distilled water as the test solution. Section D reports the cultural data applicable to each growth series, such as the time of the experiment, the soil used, and the crop planted. Every reasonable effort was made to keep growth conditions uniform for all cultures in each series. After growth had started and stands had been secured, the pots in

each series were randomized, and the customary care was given. Tap water was used for watering the crops.

The growth series numbered 1a as reported in table 1 inadvertently lacked columns percolated with distilled water. The same soil was used as in series 1, and the growth periods overlapped. No great error is involved, the writer believes, in using the yield figures for distilled-water columns from series 1 to evaluate the results secured for $\text{Ca}(\text{NO}_3)_2$ and for urea.

In the various growth series, the amount of nitrogen applied per column varied somewhat. In growth series 1, each column received 30 milligram-atoms of nitrogen; in series 1a, 12.4; in series 2, 30 and in each series from 3 to 7 inclusive, 10 milligram-atoms of nitrogen per column.

Besides the yields of the different pots in the columns resulting from the different solutions, table 1 reports yields from control pots grown simultaneously. Each of these received one-third the volume of the same concentration of the test solution in question as was added to the respective column of three pots. The various levels of statistical significance for each of the values are expressed as superscripts given in footnotes.

CHEMICAL TESTS

Some of the growth studies reported in table 1 gave inconclusive evidence with certain compounds. During the revision of the manuscript, it seemed desirable to make a limited number of tests (by methods employed in other studies) with some of the more interesting solutions. Each solution to be tested was added to a single pot containing 400 gm. of dry soil (C-68) in four successive portions applied about 2 hours apart. The first portion, 250 ml., gave about 90 ml. of percolate. The other three portions were of 115 ml. each. Suitable aliquots of the original solution and successive percolates were analyzed for nitrogen by the Kjeldahl method and the concentration of nitrogen in milligram-atoms per liter calculated. The results are shown in table 2.

TABLE 2.—*Concentration of nitrogen as determined by the Kjeldahl method in the original solution and in four successive percolates of approximately 100 ml. each passing through Yolo fine sandy loam*

Solution	Concentration of nitrogen per liter in—				
	Original solution	Percolate 1	Percolate 2	Percolate 3	Percolate 4
	Milligram-atoms	Milligram-atoms	Milligram-atoms	Milligram-atoms	Milligram-atoms
Distilled water.....	0.0	0.8	0.4	0.4	0.2
Guanidine carbonate.....	26.0	1.2	.2	.4	.4
Cyanuric acid.....	19.4	13.2	18.2	19.0	19.2
Dicyandiamide.....	19.4	13.8	18.8	20.4
Egg albumin.....	17.0	1.2	1.4	3.2	5.0
Do.....	46.2	1.8	5.4	12.6	19.6

NITROGEN IN ANIONIC UNITS

The soils in this study that were stimulated in yield of plants by $\text{Ca}(\text{NO}_3)_2$, NaNO_3 , NaNO_2 , KCN (fig. 1, *F*), and KCNO (fig. 1, *G*)

did not retain the nitrogen of any of these compounds sufficiently to affect significantly the yields of the subsequent crops of sorghum. Sulfamic acid, picric acid, and NaCNS, being toxic, significantly decreased the yields of these crops and were not retained significantly by these soils. The yellow color of the picric acid solution was easily recognized in the leachings from the bottom pot. Neither potassium ferrocyanide nor ferricyanide significantly affected the yields of sorghums in these studies. Judging from the yellow color of the leachings, the soil did not retain the whole of either of these compounds. As is widely recognized, calcium cyanamide rapidly hydrolyzes in the soil to give urea, which in turn is subject to further transformations. Perhaps because of the small number of replicates used, statistically significant differences in yield were not secured between successive pots of the CaCN_2 columns (fig. 1, A). The yields of the top pots are, however, significantly (P lies between 0.02 and 0.05) greater than those of the bottom ones. This is the same type of behavior secured with urea as reported in section C, growth series 3.

NITROGEN IN CATIONIC UNITS

In section B of table 1 are reported the results with solutions containing nitrogen in cationic units. Practically without exception, all the nitrogen present in these units was retained in the top pots of their respective series. With NH_4OH , the increases in the second pots just reached the lowest level of statistical significance. Possibly some NH_3 gas from NH_4OH diffused ahead of the percolating solution. The ammonium nitrogen of all other compounds tested was retained in their respective top pots.

These cultures demonstrate in plant growth what Way, as cited by Russell (12, p. 148), showed chemically in 1850. The results with ammonium sulfamate show the beneficial effects of the ammonium ions retained in the top pot superimposed on the toxic effects of the sulfamate ions, which, though not retained by the soil solids, were (sulfamic acid, sec. A) generally present throughout the column in the soil solution.

Since hydrazine as listed by Hodgman (8) is a base not much weaker than ammonia, its retention by the soil would be expected. Hydroxylamine, according to Ölander (11), has a dissociation constant of 1.07×10^{-8} as a base; evidently it is somewhat weaker than hydrazine, though not sufficiently so to be classed as very weak. Except for semicarbazide, it is probably the weakest base listed in section B.

Results similar to those with ammonium salts were secured with the various methyl-substituted ammonium compounds; the hydrochlorides of methylamine, dimethylamine, and trimethylamine; and tetramethylammonium chloride. Cultures of some of these are shown in figure 2. The nitrogen of choline, a basic compound occurring in some plants and resulting from the hydrolysis of the lecithins, was retained in the top pot.

Arginine, generally classed as a diamino-monocarboxylic amino acid, has moderately strong basic properties. In consequence, its complete retention in the top pot of the column is not surprising.

Guanidine, a monoacidic base, is almost as strong as sodium hydroxide (13). We should, therefore expect it to be retained completely in the top pot of the column. Since there was no growth in the top pot of the column, evidently the guanidine, which is toxic, had been retained. Data in table 2 likewise confirm this retention. In addition, apparently, some transformation products, water-soluble and nitrogen-containing, were formed during percolation and were carried down in the percolating waters, causing significantly

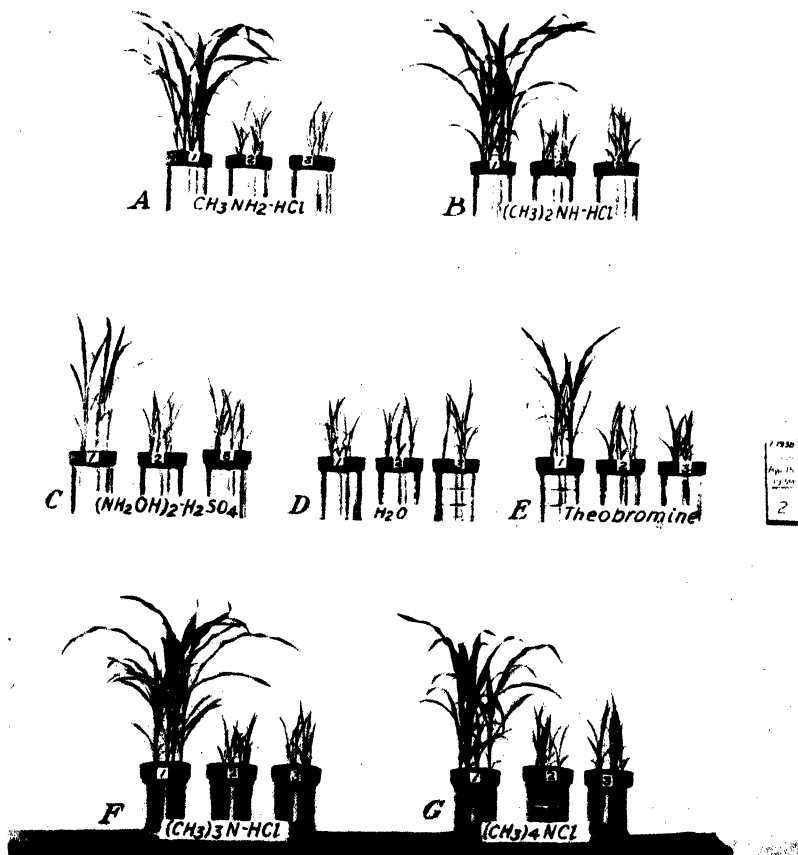


FIGURE 2.—Effect on plants of the complete retention of nitrogen from percolating solutions of various compounds by the soil in the top pots of the respective columns: A, B, F, and G, Methyl-substituted ammonium compounds; C, hydroxylamine sulfate, D, distilled water; E, theobromine.

increased growth in the second pot. Semicarbazide (8) is a weak base; most of its nitrogen was retained in the top pot. The significantly increased growth of the second and third pots over those of the check would indicate, however, either an incomplete retention of semicarbazide, or the movement of some of its decomposition products down in the column.

If a dilute solution of NH_4Cl is shaken up with a portion of soil and then filtered off, and successively shaken up and filtered off, each time with a new portion of the same soil, the concentration of NH_4 ions rapidly diminishes with each successive transfer, until it has practically disappeared from solution. The NH_4 ions have replaced an equivalent amount of other cations such as Ca, Mg, Na, and K and have thereby become attached to the solid phase of the soil. If the amount of solution and the soil be reduced to very small but still finite quantities with the concentrations and ratios remaining constant, the same type of results would be expected.

The layers of soil in the percolating column in order from top to bottom may be regarded as successive portions of the same soil discussed above, arranged naturally so that the filtrate from one automatically comes in contact with and percolates through the one below it by the action of gravity. As the front of the percolating solution of NH_4Cl advances downward through the soil, it may be considered to approach equilibrium more or less completely with each layer in turn. Even if equilibrium is by no means completely attained, still each successive layer of soil will exchange Ca, Mg, Na, and K ions for the NH_4 ions from the percolating liquid as it trickles by. Each successive addition of solution upon passing down through the soil will be higher in concentration of NH_4 ions than its predecessor; hence its original supply of NH_4 ions will penetrate farther down in the soil column. The exchange capacity of the average soil for NH_4 ions is so great and the speed of reaction so rapid, however, that no significant quantities of NH_4 ions would ordinarily leave the top pot of a three-pot column.

NITROGEN IN AMPHOTERIC OR APPROXIMATELY NEUTRAL UNITS

A soil containing 150 milliequivalents of replaceable cations per kilogram may be considered to be fairly well buffered against material change in reaction from solutions of amino acids containing 20 milligram-atoms of nitrogen per liter, especially when the amounts of the solutions are but little more than enough to wet the soil thoroughly. The reaction of the soil through which the solution of the amino acid is percolated is one of the dominating factors in determining the possible chemical reactions of these amphoteric substances. The soils used in these studies were approximately neutral to slightly alkaline.

An amino acid such as arginine with a basic isoelectric point (pI^3 about 10.8) would be expected to dissociate, so that in a neutral soil (about pH 7) its nitrogen-containing ions would be cations. The complete retention of the nitrogen of arginine in the top pot as reported in section B, table 1, was therefore not surprising. A dicarboxylic monoamino acid such as glutamic acid (pI^3 about 3.2) in a neutral soil would be expected to have its nitrogen in anionic units. Though its nitrogen penetrated to a considerable extent into the middle and bottom pots, yet penetration was not the same as with the nitrogen of other anionic units, such as nitrate, nitrite, cyanate, sulfamate, etc. What other properties of glutamic acid are responsible for a greater retention of its nitrogen in the top pot remains to be determined.

³ pI is the pH of the isoelectric point.

Glycine (pI about 6.0) and cysteine (pI about 5.1) with isoelectric points lying in between those of arginine and glutamic acid were also between them in the ability of the soil to retain the nitrogen of each. Cultures showing the results of tests with some of these compounds appear in figure 3.

Though the growth response in table 1, section C, showed neither benefit nor injury either from cyanuric acid or from diacyandiamide, the chemical data of table 2 clearly indicate that only a very little of the nitrogen of these compounds was retained by the soil.

The nitrogen of formamide was retained rather strongly (table 1, sec. C), but still incompletely by the soil of the top pot. Under the conditions of these experiments, there is no evidence of retention of

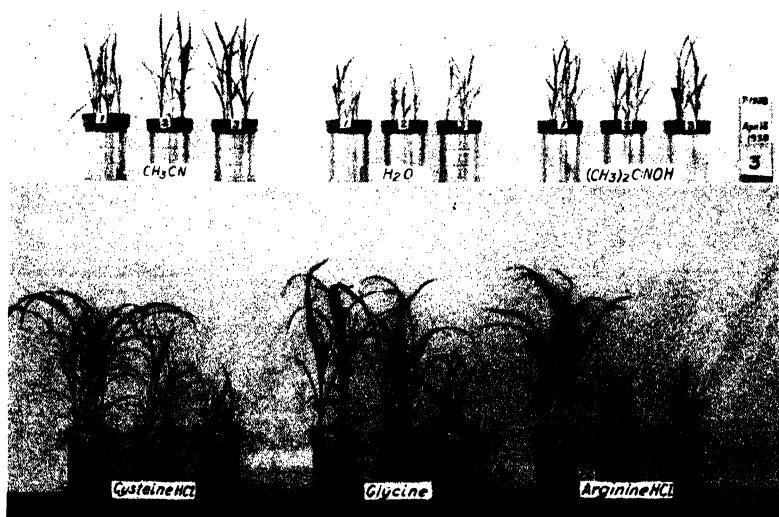


FIGURE 3.—Types of plant response to and retention by soil of various nitrogenous organic compounds when used in percolating solutions. No retention with acetonitrile (CH_3CN); complete retention with arginine hydrochloride. Intermediate types of retention with the amino acids glycine and cysteine. Acetoxime ($(\text{CH}_3)_2\text{C}:\text{NOH}$, gave but little response and no evidence of retention.

the nitrogen of acetamide, the yields from the three pots of the column being nearly equal (table 1, sec. C). Conant (1) regards amides as essentially neutral substances, while Sidgwick (13) regards them as very weak bases. Hodgman (8) lists acetamide as a base, with, however, a dissociation constant less than that of water. The equivalent conductance as determined (10, v. 6, pp. 261–262) at about the same concentrations is slightly more than 10 times as great for acetamide as for formamide. Assuming from these data that formamide is a weaker base than acetamide, the difference in retention by the soil solids between these two amides could not well be explained on the basis of their basic and acidic properties.

For growth series 1a (table 1, sec. C), the trials with urea showed definite and statistically significant but not strong retention of the nitrogen by the soil in the successive pots, beginning at the top. In series 3, although the differences in yields of consecutive pots in the

column are not significant, the yields of the top pots are significantly ($P=0.02$) greater than those of the bottom ones. Some other trials with urea conducted over the last 2 years have suggested the same type of behavior; but insufficient numbers of replicates and high variability in yields have resulted in inadequate statistical significance. It was hoped that the various derivatives of urea tested would show how the substitution of various organic radicals into the molecule affects the retention of urea by the soil, but with the variability encountered, three replications have been insufficient to give even suggestions. Thiourea proved toxic throughout the soil column.

Acetonitrile and acetoxime gave no evidence of being retained by the solids of the soil. With the acetonitrile, in fact, an anomalous situation occurred. A significantly greater yield was secured from the bottom pots than from the middle or top ones.

Peptones, essentially a mixture of polypeptides, might be expected to react as do amino acids in these experiments. The evidence so far gathered and herein reported agrees qualitatively with such an hypothesis. More extended trials will be necessary to determine whether the apparent movement of nitrogen compounds to the second and bottom pots is significant.

The yields of successive pots of the egg-albumin columns are not significantly different. Those of the top pots exceed those of the bottom pots by differences that are highly significant. The chemical tests reported in table 2 clearly show that most of the nitrogen of egg albumin was retained in the top pots. The amounts percolating through the soil, especially in the last percolate, are appreciable, however.

It has been suggested (3) that one possible interaction of gelatin with the soil is a filtering out of the dispersed colloid from the percolating solution by the soil as an ultra filter. In a soil near neutrality both gelatin and the soil particles would be negatively charged. In consequence, maximum adsorption of the gelatin by the soil because of neutralizing opposite charges would not occur. Giesecking (6) lists "gelatine" among the exchangeable cations that react with montmorillonite and related minerals. As he used clays saturated with hydrogen ions, his findings might conceivably not apply to the more alkaline conditions near neutrality prevailing in these tests. Other factors undoubtedly contribute to the results observed.

The nitrogen of theobromine (dimethyl xanthine, a purine base) was completely retained in the top pot. Hodgman (8) lists caffeine (trimethyl xanthine) as being little stronger as a base than water. Assuming that there is small difference between these two compounds as bases, the complete retention of the nitrogen of theobromine in the top pot is somewhat surprising if considered from the viewpoint of its basic properties alone. Some unknown substance or substances in the soil solution might have precipitated the theobromine, or the soil solids might have reacted with it in such a way as to remove it from solution.

DISCUSSION

The nonretention of nitrogen in anionic units is not unexpected; and from the standpoint of the theory of cationic exchange in soils, the practically complete retention of the nitrogen in medium to strongly

basic cations is almost predictable. Considering, however, the retention of the nitrogen of the approximately neutral or amphoteric units, the behavior of any compound cannot be predicted on the basis of reaction alone. Thus though formamide is probably a weaker base than acetamide, its nitrogen was retained much more strongly by the soil solids than that of acetamide.

In the presence of proper catalysts or under conditions favoring microbial activity, ammonia may be more or less easily split off from these parent compounds. Sidgwick (13, p. 141) indicates that formamide is more easily hydrolyzed to ammonia and the corresponding organic acid than is acetamide. If ammonia were thus separated from any of these compounds during percolation, the soil would react with it in accordance with its own individual chemical properties and would retain it at, or very near, the point in the soil where that particular molecule of ammonia was split off. If such were the mechanism involved, a slower rate of percolation would allow a greater lapse of time for catalytic reaction or biological activity to become effective in splitting off more ammonia.

A reexamination of yield data disclosed approximately equal sums for the yields of three pots for given columns percolated with solutions of the same compound. Considerable variation occurred, however, among yields secured from the replications of the first, second, or third pots respectively from these columns. Chance variations in the rate of dripping from the reservoir above, together with unequal rates of percolation from pot to pot caused by chance differences in packing of the soil, might well have produced such variation. The writer considers this unequal rate of percolation as one major cause of high variability in some of these tests. In growth series 7, glycine, cysteine hydrochloride, and arginine hydrochloride were percolated by adding in five successive portions approximately one-fifth of the percolating solutions to the respective reservoirs, with about 12 hours between successive applications. Prolonging the time of percolation, as in growth series 7, apparently favored ammonium formation by catalytic reaction or microbial activity. See glycine in growth series 3 and 7, table 1, sec. C.)

Transformations of cysteine, which is very soluble, to cystine, which is very slightly soluble, may take place by oxidation in air or under alkaline conditions (7, p. 123). With the concentration used (approximately 20 millimols per liter), about 90 percent of the nitrogen present would be precipitated if all the cysteine were changed to cystine. This transformation might well be one of the mechanisms by which such a large part of the nitrogen of cysteine was retained in the top pot. No mechanism of the same type seems to be at hand to explain the somewhat similar retention with glycine.

In case injury followed a particular test, it was assumed that the compound in question was toxic, although the damage may have been caused by decomposition products of that compound. Since the soils used were deficient in nitrogen under the conditions of the experiment, significant beneficial responses showed the position of the nitrogen of the compound regardless of whether this element was present in its original form or in physiologically effective forms resulting from subsequent biological changes.

It is assumed that many of these compounds if left to incubate a sufficiently long time would be converted almost quantitatively to nitrates. Such nitrates would, of course, be expected to react as have the nitrates reported in this paper.

Retention of the soil solids of some of the compounds tested, such as potassium ferrocyanide, potassium ferricyanide, and phenylurea as shown in table 1 was not fully determined by the criterion of subsequent plant response. Obviously these compounds were neither toxic enough nor beneficial enough in these experiments to influence the crop. Studies by the methods of analytical chemistry would undoubtedly be required for further advancement of our knowledge in this direction.

This study has served to give in terms of plant response a qualitative (and in some cases a semiquantitative) estimate of the reaction of the nitrogen of a wide variety of compounds in solution with the solid phase of the soil. Further elucidation of the role of various factors involved, especially with some of the compounds presenting new types of behavior, must await the accumulation of further experimental evidence.

SUMMARY

Each nitrogen compound in solution was allowed to percolate through a column of three pots containing dry soil deficient in nitrogen. The volume of solution was sufficient to wet all the soil but without great excess. If the compound was beneficial, the enhanced growth of the subsequent test crop was used to determine retention; if it was toxic, the reduced growth was used. The results obtained were as follows:

(1) Nitrogen contained in anionic units in the compounds: Calcium nitrate, sodium nitrate, sodium nitrite, potassium cyanide, and potassium cyanate were not retained by the solids in the soils tested, as was shown by beneficial plant response in all pots of the respective columns. Nonretention of sulfamic acid, picric acid, and sodium thiocyanate was shown by injury to the growth throughout the pots of the respective columns.

(2) Nitrogen contained in cationic units in the compounds: Ammonium acetate, ammonium carbonate, ammonium hydroxide, ammonium phosphate (monohydrogen), ammonium sulfamate, ammonium sulfate, hydrazine sulfate, hydroxylamine sulfate, tetramethylammonium chloride, and the hydrochlorides of trimethylamine, dimethylamine, methylamine, choline, and arginine were completely or almost completely retained in the top pots of their respective columns. Most of the nitrogen of semicarbazide hydrochloride and of guanidine carbonate was retained in the top pots of their respective columns; yet small but still significant amounts penetrated to lower pots.

(3) There was no evidence of any retention of the nitrogen of acetonitrile, acetoxime, or acetamide. The nitrogen of glycine, cysteine hydrochloride, glutamic acid, formamide, urea, and CaCN_2 was retained to a certain degree by the soil solids. Indications are that decomposition to liberate ammonia and the resultant retention of this form of nitrogen during the period of actual percolation are important factors in retention with these compounds. Peptone, egg

albumin, and blood albumin showed the same type of retention, whereas the nitrogen of gelatin and that of theobromine were completely retained in the top pots of their respective columns.

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THE RESISTANCE OF PROGENY OF KATAHDIN POTATOES TO VIROSES ¹

By LEON K. JONES, *associate plant pathologist*, C. L. VINCENT, *associate horticulturist*, and EARL F. BURK, *formerly assistant horticulturist*, Washington Agricultural Experiment Station ²

INTRODUCTION

Schultz et al. (11) ³ reported the Katahdin variety of potato (*Solanum tuberosum* L.) as being resistant to infection in the field by the mild mosaic virus and by the latent or X virus. They further reported the Katahdin variety to be resistant to the latent virus when mechanical inoculation methods were used, but susceptible when tested by tuber grafts. Smith (14), however, reported that Katahdin is not resistant to the X and Y viruses when inoculated by the rubbing and needle-scratch methods. In a previous publication (9) it was shown that Katahdin, as well as Chippewa and Russet Burbank, was somewhat resistant to infection by the Y virus in the field when compared with Bliss Triumph, Gold Coin, Irish Cobbler, Warba, and U. S. D. A. 41956. It was further shown that Katahdin was the only variety that transmitted to seedling progeny resistance to infection by the Y virus in field tests of seedlings developed from crossing and selfing 11 varieties of potatoes.

The Y virus in combination with the X virus is responsible for the rugose mosaic disease that is very destructive, in many potato-growing areas, to common commercial varieties of potatoes. Since the older commercial varieties in the United States are carriers of the X virus without material damage to the stock, the introduction of the Y virus is the destructive element in rugose mosaic (6).

It has been shown in a previous publication (9), as well as by other investigators, that the Y virus is capable of causing severe damage to potatoes even in the absence of the X virus. This has been demonstrated by inoculation of the Y virus into seedlings that were virus-free. Marked variation in symptoms, including mottling and severe necrosis, is exhibited by seedlings and clones infected only by the Y virus (9).

In order to obtain further information on the value of Katahdin as a parent in the development of potato seedlings resistant to viroses, a number of tests were made beyond those reported in 1937 (9). The results of these tests are reported in the present paper.

RESISTANCE TO VEIN-BANDING

METHODS

Seed developed by the Division of Horticulture of the State College of Washington, as well as seed and tubers of named and numbered varieties furnished by the United States Department of Agriculture, were used in these investigations.

¹ Received for publication August 7, 1939. Published as Scientific Paper No. 413, College of Agriculture and Experiment Station, State College of Washington.

² Acknowledgement is made to Dr. F. J. Stevenson of Bureau of Plant Industry, U. S. Department of Agriculture, for furnishing seed and tubers of named and numbered varieties for the tests.

³ Italic numbers in parentheses refer to Literature Cited, p. 644.

The seed was planted in the greenhouse about March 15 each season and the young seedlings transplanted to beds 4 to 6 weeks after seeding. Previous to 1938 all the seedlings were transplanted to the field during the last of May or the first part of June. In 1938 and 1939 the seedlings were mechanically inoculated with the Y virus according to the method described by Jones (8) soon after they were transplanted to the beds in the greenhouse. A period of 30 to 45 days was allowed following inoculation before roguing the affected plants and transplanting the symptomless (resistant) plants to the field.

The year the seedlings were started and the numbers observed are as follows: 1924, 167; 1925, 28; 1926, 138; 1927, 924; 1928, 443; 1929, 448; 1930, 449; 1931, 350; 1932, 720; 1934, 2,081; 1936, 776; 1937, 4,000; 1938, 9,728; and 1939, 8,038. Records of the prevalence of vein-banding (Y virus) in the plantings have been taken each year since 1933 by making three field inspections during the growing season. Previous to 1933 the badly diseased stock was rogued from the planting without definite information on the causal virus. During the winters of 1933 to 1937 all the virus-free clones, those recorded as "virus infection questionable," and many of the virus-infected clones, as recorded in the previous summer observations, were indexed in the greenhouse. Five to twenty tubers representing each clone to be tested were planted in the greenhouse. The indexing consisted in observing the symptoms of disease, if any, shown by each plant, and inoculating with macerated leaf tissue from each potato plant into tobacco plants according to the method described by Jones (8). These detailed studies in the greenhouse showed that the Y virus was responsible for practically all of the viroses observed in the field plantings with the exception of a few cases of leaf roll and latent mosaic. In 1938 and 1939 curly top developed in the plantings to a damaging extent.

In addition to the seedlings started each year, field plantings were made with the clones noted the previous year as resistant to the Y virus. Clones that had been grown in the field 2 or more years were planted with 25 hills per numbered variety, often in triplicate, and the clones that had been grown only 1 year from true seed were planted with 12 hills per numbered variety. Many of the numbered varieties noted as susceptible to vein-banding, but having desirable characters, were saved each season and interplanted in the rows with the resistant varieties. Twenty-five hills each of the named and numbered varieties, often in triplicate, and the single drop tubers (1 small tuber from each numbered variety) furnished by the United States Department of Agriculture were interplanted in rows with the stock developed from seed.

Most seasons every third or fifth row was planted with varieties known to be affected with vein-banding, and from 40 to 50 percent of the stock used each season was affected with vein-banding. Although the resistant clones and varieties were not uniformly interplanted with the diseased stock in the rows, observations each season showed that ample opportunity was afforded for 100-percent infection of the susceptible clones and numbered varieties.

Varieties which showed no vein-banding or less than 30 percent affected in a given season were classed as resistant, in contrast to the

susceptible ones, most of which showed 100-percent infection under similar conditions.

EFFECT OF A COMBINATION OF VIRUSES

In order to determine whether a combination of viruses might be responsible for the variation in symptoms, a series of tests was made on different lots of seedlings using the virus extract from necrotic (fig. 1) as well as from mottled (fig. 2, *C*) plants.



FIGURE 1.—Necrosis of Katahdin, selfed seedlings produced by inoculation with the Y virus.

TABLE 1.—*Variation of symptoms in potato seedlings following mechanical inoculation with the Y virus*

Source of virus	Parentage of seedlings inoculated	Seedlings inoculated	Plants infected that showed—	
			Mottling	Necrosis
		Number	Percent	Percent
Mottled seedling in field ¹	Early Norther × McCormick.....	103	91.5	6.2
	Katahdin, selfed.....	103	18.4	13.6
	Early Norther × McCormick.....	36	91.7	8.3
Necrotic Katahdin seedling ²	Keeper, selfed.....	16	100.0	0
	Irish Cobbler × Keeper.....	40	100.0	0
	Katahdin, selfed.....	45	35.6	33.4
Mottled Katahdin seedling ³	Early Norther × McCormick.....	38	92.1	7.9
	Keeper, selfed.....	15	100.0	0
	Irish Cobbler × Keeper.....	43	100.0	0
	Katahdin, selfed.....	45	35.6	28.8

¹ The Y virus was obtained from infected tobacco plants that had been inoculated from seedling 2563 tissue collected in the field.

² The Y virus was transferred from necrotic Katahdin seedlings noted above.

³ The Y virus was transferred from mottled Katahdin seedlings of second test noted above.

An analysis of the results of these tests (table 1) shows that the ratio of necrosis to mottling was practically the same in infected plants of seedling lots regardless of whether the inoculum was from necrotic or mottled plants. This shows that a single Y virus was responsible for the variable symptoms observed.



FIGURE 2.—Symptoms produced on potato seedlings: *A*, Mottling; *B*, necrosis by the tobacco mosaic virus; *C*, mottling by the Y virus.

TABLE 2.—The effect of vein-banding upon yields of potato varieties, Pullman, Wash., 1938

Variety	First year in field ¹			Second year in field ²		
	Hills observed	Average yield per hill		Hills observed	Average yield per hill	
		Total	U. S. No. 1		Total	U. S. No. 1
	Number	Pounds	Pounds	Number	Pounds	Pounds
U. S. D. A. 46000.....	71	2.0	1.7	69	0.5	0.4
Earlaine.....	64	2.4	2.0	53	1.0	.7
Houma.....	64	2.5	1.9	70	.7	.3
Katahdin.....	69	3.8	2.9	55	3.6	2.7

¹ New stock obtained from the U. S. Department of Agriculture for 1938 planting. Field records early in the growing season showed no vein-banding.

² Stock saved from 1937 trial plots in Pullman. The Katahdin variety was carefully rogued in 1937, 35 percent of the plants having become affected with vein-banding. The other varieties became 100 percent affected with vein-banding in 1937 and were not rogued.

EFFECT OF VEIN-BANDING ON TUBER YIELDS

The effect of vein-banding on tuber yields was obtained during the season of 1938 on a number of varieties in the field planting, as shown in table 2. An analysis of the results of these tests shows that vein-banding reduced the yields of U. S. D. A. 46000, Earleine, and Houma 75, 58, and 72 percent, respectively, in the second season following 100-percent infection with the virus. Only 5-percent reduction in yield was noted in the rogued Katahdin stock.

RESISTANCE OF SEEDLINGS TO MECHANICAL INOCULATION

In order to reduce the population to be planted in the field and to determine if mechanical inoculation could be used as a criterion of susceptibility, the seedlings that were started in 1938 and 1939 were mechanically inoculated with the Y virus in the greenhouse soon after they were transplanted to the beds. A period of 30 to 45 days was allowed before roguing the affected plants and transplanting the symptomless (resistant) plants to the field. Since the susceptible plants showed symptoms in 15 to 25 days following inoculation, all plants that would develop symptoms from the mechanical inoculation probably showed the infection and were rogued in the greenhouse. Observations made 10 to 12 days after the symptomless plants were placed in the field failed to show any affected plants which indicates that only healthy plants or possibly those with masked symptoms were taken into the field.

The results recorded in table 3, where the tests are summarized according to the parents used in seed production, show that some lots of seedlings were 100 percent susceptible to mechanical inoculations with the Y virus, while others were somewhat resistant; but none of the seedling lots showed immunity to infection. Seedling lots produced from seed of which Katahdin was a parent consistently showed some resistance to infection. Based on Katahdin selfed seedlings showing 54-percent resistance, it appears that higher percentages of resistance were obtained by crossing Katahdin with Ackersegen, Imperia, Sebago, and U. S. D. A. 41956, and that reduced resistance was obtained by crossing Katahdin with Earleine-Sebec, Earleine-Charles Downing, and Houma. The use of Green Mountain, No Blight, and Albion in crosses with Katahdin did not materially change the resistance of the seedlings as compared with Katahdin, selfed seedlings.

RESISTANCE OF SEEDLINGS TO FIELD INOCULATION

The results of the field tests with seedlings (table 3) show that mechanical inoculation with the Y virus does not record the complete susceptibility of seedling lots since greater susceptibility was observed in the field beyond that noted in the greenhouse following mechanical inoculation. Mechanical inoculation in the greenhouse, however, appears to indicate the relative susceptibility of seedling lots.

Only seedlings having Katahdin as a parent showed resistance to vein-banding in the field. Crossing Katahdin with Sebago or U. S. D. A. 41956 appeared to increase the percentage of resistance over that of Katahdin, selfed seedlings, but a consideration of the standard deviation shows that these increases are not significant.

TABLE 3.—Summary of tests to determine the resistance of potato seedlings to vein-banding ¹

Parentage of seedlings	Seed lots tested	Seedlings and clones observed ²	Seedlings inoculated in the greenhouse ³		Clones resistant in the field ⁴	Clones rogued for curly top ⁵
			Observed	Resistant		
	Number	Number	Number	Percent	Percent	Percent
McCormick:						
Selfed	1	65			0.0	
Early Norther	11	1,100	177	4.0	.0	
Keeper	4	131			.0	
Keeper, Early Norther	2	117			.0	
Keeper:						
Selfed	3	466	131	.0	.0	
Jersey Red Skin	1	85			.0	
American Wonder	3	100			.0	
Cobbler	3	252	183	.0	.0	
Burbank, selfed	9	1,342			.0	
Cobbler, selfed	4	470			.0	
Earlaine:						
Selfed	3	1,077	1,077	.0		
U. S. D. A. 41956	1	870	870	54.4	.0	20.1
No Blight	1	(360)	360	43.4	(.0)	17.3
Late Cobbler	1	61			.0	
Hindenburg, Ostragis	1	(198)	198	55.4	(.0)	6.9
Katahdin:						
Selfed	28	4,754 (180)	2,739	⁶ 54.1±6.8	⁶ 4.4±2.68	⁶ 32.4±21.0
Green Mountain	4	3,765 (1,033)	3,765	44.5	2.8 (0)	13.8
No Blight	7	1,568 (576)	813	58.5	4.9 (2.3)	20.4
Sebago	2	612 (72)	612	74.8	8.2 (1.4)	59.1
U. S. D. A. 41956	4	1,145 (354)	1,145	72.6	7.4 (4.2)	35.0
Houma	2	1,740 (540)	1,740	27.4	1.2 (7)	10.1
Aekersogen	1	(58)	58	74.0	(.0)	.0
Imperia	1	(493)	493	82.8	(3.4)	34.8
Albion	1	(473)	473	55.2	(.8)	16.8
Earlaine, Sebec	2	455 (303)	455	29.0	.0 (0)	20.0
Earlaine, Charles Downing	1	(432)	432	5.3	(.0)	38.9
Carmen No. 3, Bussola, Rural						
New Yorker No. 2	7	2,766 (1,598)	2,695	13.8	2.3 (0)	36.1
Yellow-fleshed variety from Costa Rica, Arrostook Wonder, Sutton Flourball, Rural New Yorker No. 2, Bussola	6	84	84	48.8	4.7	18.2

¹ The following crosses with than 50 seedlings in each lot were also observed in the field and failed to show any clones resistant to vein-banding. McCormick crossed with American Wonder, Beauty of Hebron, Burbank, Early Norther, Early Six Weeks, Irish Cobbler, Jersey Red Skin, Keeper, Late Rose, McKinley, and Mortgage Lifter. Keeper crossed with Burbank. Late Rose, selfed, and crossed with American Wonder and Burbank. Early Norther, selfed. Katahdin crossed with American Wonder, Burbank, Chippewa, Early Norther, Eureka, Irish Cobbler, Keeper, and McCormick.

² Figures in parentheses show data collected on tests made for only 1 season. All other figures indicate that data are summaries of observations through 2 or 3 seasons.

³ The seedlings were mechanically inoculated with the vein-banding virus in the greenhouse. A period of 30 to 45 days was allowed following inoculation before roguing the diseased plants and transplanting the symptomless (resistant) plants to the field.

⁴ Clones showing less than 30 percent of infection were classed as resistant, and the percentage of clones resistant in the field was calculated on the total number of seedlings and clones of each parentage that was observed in the greenhouse and the field.

⁵ Curly top caused by the leafhopper-transmitted virus that is associated with curly top of beets became a factor in the experimental plantings in 1938 and 1939. The percentage of clones rogued for curly top was calculated on the number of clones exposed to infection in the field. Lack of data shows that the tests were made previous to 1938 before curly top appeared as an important factor in the experimental plantings.

⁶ Standard deviation of the mean. $S. D. = \sqrt{\frac{\sum d^2}{n-1}}$.

As was shown in the mechanical-inoculation tests, Earlaine-Sebec and Earlaine-Charles Downing crossed with Katahdin reduced the resistance of the seedlings in comparison with that obtained in seedlings from Katahdin, selfed parentage.

An example of the resistance shown in the field tests may be stated as follows: Of the 776 seedlings started in 1936, 89 were saved as resistant or promising and grown again in the field in 1937. The observations in the field in 1937, as well as indexing 5 tubers per clone

in the greenhouse during the winter of 1937-38, showed that only 18 of the 89 clones continued to show high resistance to vein-banding. The 18 resistant clones were grown in the field in 1938 and again in 1939 and were carefully rogued each season. Field observations on the 18 varieties during the last 2 seasons have shown vein-banding as follows: In 1938, 7 were virus-free, 6 showed less than 10 percent, and 5 showed less than 30 percent of the plants infected; in 1939, 7 were virus-free, 5 showed less than 10 percent, and 6 showed less than 40 percent of infection.

RESISTANCE OF POTATO VARIETIES TO FIELD INOCULATION

A quantity of tubers, as well as single drops of named and numbered varieties of potatoes recently developed by the United States Department of Agriculture, were planted in the same planting with the seedlings. If a quantity of the tubers of the varieties was furnished, 25 hills per variety were planted in triplicate. The following varieties became 100 percent affected with vein-banding in 2 years: Earleine, Golden, Houma, 41956, 46000, 46110, 46125, 44537, 47154, and 47160. Under similar conditions the percentage of infection for the following varieties was: 46842, 0; 47208, 0; Chippewa, 1.9; 47196, 2.8; Katahdin, 12.9; 47296, 13.2; 336-123, 19.4; and Sebago, 31.5.

The results of tests with single drop tubers of numbered varieties recorded in table 4 show that selfing Earleine or crossing Earleine with Katahdin or U. S. D. A. 41956 gave seedlings of reduced resistance as compared with seedlings produced from selfing Katahdin or crossing Katahdin with No Blight or U. S. D. A. 41956. These results are in accord with those obtained by mechanical inoculation and by field tests with seedlings.

SEED TRANSMISSION OF THE Y VIRUS

Observations on the seedlings produced in the greenhouse (tables 1 and 3), many of which were grown from seed collected from vein-banding-affected plants, failed to show any indication that the Y virus is seed transmitted.

TABLE 4.—*Tests with single drops of numbered varieties to determine resistance to vein-banding, Pullman, Wash., 1939*

Percentage of single-drop tubers ¹	Hills observed	Hills virus-free ²
	Number	Percent
Earleine, selfed.....	29	0.0
Earleine × U. S. D. A. 41956.....	32	3.1
Earleine × Katahdin.....	80	5.0
Katahdin, selfed.....	37	18.1
Katahdin × No Blight.....	88	21.6
Katahdin × U. S. D. A. 41956.....	74	25.7

¹ Single-drop tubers of numbered varieties furnished by the U. S. Department of Agriculture.

² Planting observed and rogued 3 times during growing season. The last observation was made Oct. 6, 1939, 3 days before harvest.

RESISTANCE TO CURLY TOP

A serious disease appeared in the potato planting in 1938 and 1939 which showed as dwarfing and erectness of the plants, rolling, harsh-

ness, and reddening of the foliage, and extreme dwarfing and increase in the number of tubers (fig. 3). The disease was very severe in the seedling planting, often killing the affected plants and making it



FIGURE 3.—Curly top of Katahdin, selfed seedling from experimental plot, 1939.

difficult to obtain definite data on susceptibility to vein-banding. The general appearance of the disease resembled that associated with psyllid yellows or aster yellows, but frequent observations and the collection of prevalent insects failed to show the presence of the insect

vectors of these two diseases. The fact that aster yellows has never been recorded in the immediate vicinity of Pullman is further evidence that the aster yellows virus was not the causal agent.

The prevalence of curly top in tomato plantings adjacent to the potatoes and the heavy infestation of *Eutettix tenellus* (Baker) on the potato plants indicated that the curly top virus was responsible for the symptoms. The virosis known as curly top of beet, which is transmitted by the leafhopper *E. tenellus*, has been reported on potato by Severin (13) who states: "Further experiments will show whether tuber transmission of curly top occurs in potato."

Tubers collected in the field from 25 potato plants affected with curly top were planted in the greenhouse in the winter of 1938-39. This stock produced 3 plants showing spindly sprouts, erectness, inward rolling, and yellowing of foliage which indicates only a low percentage of tuber transmission. Healthy tomato scions were grafted onto the affected potato plants, and typical symptoms of the common tomato curly-top disease developed on the tomato.

The data collected on the prevalence of curly top in the seedlings (table 3) show wide variation; however, the large standard deviation of the mean indicates no significant differences in susceptibility of the seedling lots.

Curly top of tomatoes has been prevalent adjacent to the potato plots each year since the potato project was started. Observations in commercial and garden plantings of the Katahdin variety in the vicinity of Pullman, in 1938 and 1939, showed 8 to 10 percent of the plants affected with curly top. The fact that the disease was not important in the potato planting until 1938 and 1939 coincident with the general use of Katahdin as a parent in the seedlings would indicate that possibly Katahdin and Katahdin progeny are more susceptible than other varieties.

RESISTANCE TO TOBACCO MOSIAC

Allard (1), Dickson (4), Clinton (3) and Schultz and Folsom (12) have reported unsuccessful attempts to infect potatoes with the tobacco mosaic virus. Johnson (7) obtained local necrotic lesions on the inoculated leaves of Bliss Triumph but concluded that tobacco mosaic did not become systemic in the potato. Fernow (5) reported symptoms on potato closely parallel to those of streak of potato and obtained systemic infection with tobacco mosaic on the Green Mountain variety. Blodgett (2) showed that tobacco mosaic produced different symptoms on certain varieties of potatoes, the Rural New Yorker No. 9 being especially susceptible, and that on some varieties already affected with the latent virus, streak symptoms appeared. Folsom and Bonde (6) have shown that tobacco mosaic can infect Green Mountain potato plants, producing symptoms rather like those of the potato streak disease.

The susceptibility of a number of potato varieties and seedlings to infection by the tobacco mosaic virus has been determined by inoculation in the greenhouse of the State College of Washington. Tobacco mosaic virus No. 1 obtained from Dr. James Johnson of the University of Wisconsin was used in all of the tests. The virus was inoculated into tobacco plants in 1929, and the diseased plants were harvested and hung in a shed adjacent to the greenhouse. Portions of this

material which had been dried for 10 years were used to inoculate young tobacco plants, and the newly affected tobacco plant tissue was used as inoculum on the potato plants, according to the method described by Jones (8) supplemented by the sprinkling of carborundum dust on the foliage to be inoculated previous to inoculation (10).

The results of inoculations (table 5) show that plants of the Rural New Yorker No. 9 variety and seedlings produced from crosses of Early Norther \times McCormick and Irish Cobbler \times Keeper were somewhat susceptible, but that plants of the varieties Early Rose, Chippewa, Green Mountain, Irish Cobbler, Katahdin, and seedlings from selfed Earlane, selfed Katahdin, selfed Keeper, and Katahdin crossed with Houma, Sebago, or U. S. D. A. 41956 were immune to tobacco mosaic when mechanical-inoculation methods were used. The symptoms shown by potato seedlings affected with tobacco mosaic varied from distinct mottling with large, raised, dark-green areas (fig. 2, A) to extreme necrosis (fig. 2, B) depending upon the reaction of the individual susceptible seedling. Affected plants of the Rural New Yorker No. 9 variety showed leaf and stem necrosis similar to potato streak symptoms.

TABLE 5.—*Summary of tests to determine resistance of potato varieties and seedlings to tobacco mosaic*¹

Variety or parentage of seedling	Plants inoculated		Variety or parentage of seedling	Plants inoculated	
	Number	Percent		Number	Percent
Early Rose	31	0.0	Early Norther \times McCormick ..	538	19.3
Chippewa	2	.0	Irish Cobbler \times Keeper ..	179	4.5
Green Mountain	33	.0	Katahdin, selfed	444	.0
Irish Cobbler	33	.0	Katahdin \times Houma	108	.0
Katahdin	408	.0	Katahdin \times Sebago	108	.0
Rural New Yorker No. 9 ..	20	15.0	Katahdin \times U. S. D. A. 41956 ..	108	.0
Earlaine, selfed	108	0.0	Keeper, selfed ..	48	.0

¹ Tobacco mosaic virus No. 1a from tobacco plant that had been inoculated from tobacco tissue that had been dried for 10 years. An equal number of uninoculated control plants in each test failed to show symptoms of tobacco mosaic.

During the winter of 1937-38, 10 tomato scions were grafted onto 10 selfed Katahdin seedlings. The tomato scions were subsequently inoculated with tobacco mosaic. The symptoms of tobacco mosaic appeared on the tomato foliage, and definite mottling with large dark-green areas followed by necrosis of the light-green areas showed on the foliage of the potato seedlings. Inoculations from the affected potato foliage to tobacco plants showed the presence of tobacco mosaic in the potato foliage. Tubers collected from the mosaic-affected selfed Katahdin potato plants were planted in the greenhouse along with tubers collected from the Early Norther \times McCormick seedlings affected by tobacco mosaic. The plants produced from the Early Norther \times McCormick seedling tubers showed strong symptoms of tobacco mosaic but those from selfed Katahdin tubers failed to show any symptoms of the disease. Inoculations with potato plant tissue from the 2 lots of plants verified the presence of the tobacco mosaic virus in the Early Norther \times McCormick seedlings and its absence from the selfed Katahdin seedlings. These results show that the selfed Katahdin seedlings were susceptible to infection by the tobacco mosaic virus through grafts with mosaic-affected tomato scions but

that the virus was not transmitted through the tubers produced by affected plants.

RESISTANCE TO LATENT MOSAIC

Schultz et al. (11) report that leaf-rubbing, tuber-grafting, and field-contact tests failed to produce infection by the latent or X virus in the potato variety U. S. D. A. 41956. The same authors further report that Katahdin was resistant to latent mosaic in field tests, but contracted the disease in tuber-graft tests where it was expressed as top necrosis. The report of Smith (14) shows Katahdin to be susceptible to infection by the X virus by the rubbing and leaf-scratch methods of inoculation, the resultant disease showing as circular necrotic lesions followed by a mild mottling of the topmost leaves, while the other leaves developed a curious network of faint necrotic lines most pronounced on the under surfaces.

Inoculations to determine the susceptibility of Katahdin to infection by the X virus have been made at different times over a period of years. In the earlier tests with the X virus from Russet Burbank and Green Mountain plants, Katahdin was shown to be immune to infection by mechanical inoculation (table 6).

TABLE 6. --*Summary of tests to determine the resistance of Katahdin to mechanical inoculation with the latent or X virus*

Original source of the virus	Source of virus for the inoculation test	Katahdin plants in test	Plants infected by the latent virus	
			Number ¹	Symptoms
Russet Burbank	Russet Burbank	Number		
Do	Tobacco	19	0	None.
Green Mountain	do	10	0	Do.
Seedling 2277	do	10	0	Do.
Do	do	4	4	Mottling, crinkling.
Do	Katahdin	15	15	Do.
Do	<i>Datura stramonium</i>	20	20	Do.
Control	Uninoculated	24	0	None.

¹ Based on symptoms shown by each plant and inoculations to tobacco plants with potato plant tissue, alone and in combination with the vein-banding virus.

During the winter of 1933-34, four Katahdin plants were inoculated with a virus that appeared in seedling 2277, and very definite mottling of the foliage resulted (fig. 4). Further tests showed that the causal virus was definitely of the X type based on the following characters: Thermal inactivation, 68 to 70° C.; produced spot necrosis of tomato and tobacco in combination with the vein-banding virus; caused systemic infection in *Datura stramonium* (fig. 5); and retained all of its original characters after passage through *D. stramonium*.

In further tests on Katahdin this variant of the X virus consistently produced 100-percent infection in mechanical inoculations. The disease has been perpetuated through three generations of tubers and continues to show the same severe mottling without necrosis. The disease has been observed only on the experimental plants in the greenhouse. The original source of the virus is unknown beyond the fact that it appeared in plants of seedling 2277 developed from Early Norther × McCormick.

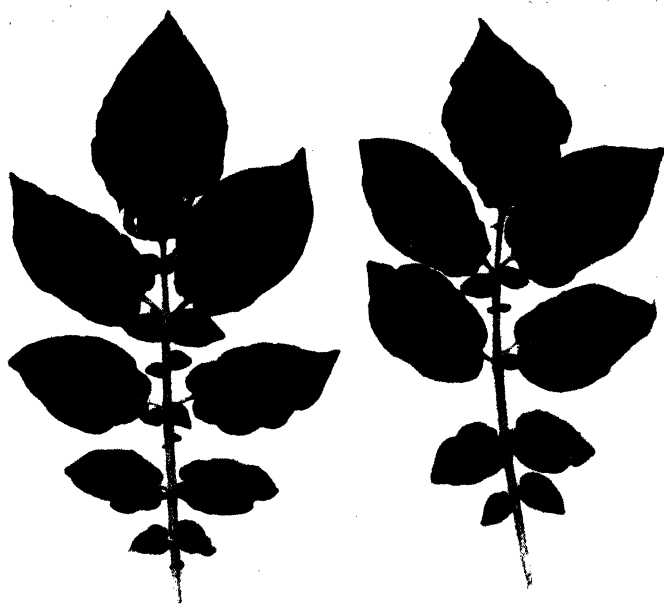


FIGURE 4.—Mottle of Katahdin leaves produced by inoculation with the X-type virus obtained from seedling 2277.



FIGURE 5.—Mottle of *Datura stramonium* (A) and tomato leaves (B) produced by inoculation with the X-type virus obtained from potato seedling 2277 compared with mottle of *D. stramonium* leaf (C) produced by inoculation with the X virus from Green Mountain potato.

The tests with the X virus, as shown in table 6, show that Katahdin is resistant to infection by the common X virus found in Russet Burbank and Green Mountain potatoes, but susceptible to the X virus from seedling 2277.

SUMMARY

The vein-banding or Y virus, which is the destructive element in rugose mosaic, produces marked variation in symptoms depending on the reaction of the individual seedling and may reduce yields of susceptible varieties as much as 75 percent.

Some lots of seedlings were 100 percent susceptible to mechanical inoculation with the Y virus, while others were somewhat resistant and none of them showed immunity. Seedling lots, produced from seed of which Katahdin was a parent, consistently showed some resistance to infection by mechanical inoculation with the Y virus; and crosses of Katahdin with Ackersegen, Imperia, Sebago, and U. S. D. A. 41956 appeared to transmit greater resistance than did selfing Katahdin.

Mechanical inoculation with the Y virus did not show the complete susceptibility of seedling lots since greater susceptibility was observed from natural inoculation in the field.

Only seedlings having Katahdin as a parent showed resistance to vein-banding in the field, and no significant increase in resistance over that shown by selfed Katahdin was obtained by crossing Katahdin with other varieties.

Many named and numbered varieties of potatoes became 100 percent affected with vein-banding in two seasons in the field, while others, including Katahdin, showed less than 32-percent infection under similar conditions.

Tests with single-drop tubers showed that selfing Earlane or crossing Earlane with Katahdin or U. S. D. A. 41956 gave seedlings with reduced resistance to the Y virus as compared with seedlings produced by selfing Katahdin or crossing Katahdin with No Blight or U. S. D. A. 41956.

Observations on thousands of seedlings produced from seed collected from vein-banding-affected plants fail to show any seed transmission of the Y virus.

The virus which causes curly top of beets was very prevalent in the field, but no significant difference in susceptibility was noted in the seedling lots.

Plants of the Rural New Yorker No. 9 variety and seedlings produced from crosses of Early Norther \times McCormick and Irish Cobbler \times Keeper were susceptible to mechanical inoculation with tobacco mosaic, but all the other varieties tested, including Katahdin and other seedlings including crosses with Katahdin, were immune.

Selfed Katahdin seedlings were susceptible to infection by the tobacco mosaic virus through grafts with mosaic-affected tomato scions, but the virus was not transmitted through tubers collected from affected plants.

Katahdin was immune to infection by mechanical inoculation with the X virus from the Russet Burbank and Green Mountain varieties, but was susceptible to a variant of the X virus obtained from potato seedling 2277.

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JOURNAL OF AGRICULTURAL RESEARCH

VOL. 60

WASHINGTON, D. C., MAY 15, 1940

21 OCT 1940
No. 10

SEASONAL DEVELOPMENT, INSECT VECTORS, AND HOST RANGE OF BACTERIAL WILT OF SWEET CORN¹

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INTRODUCTION

The work described in the present paper is a continuation of an investigation on bacterial wilt previously reported by the writers (4, 9).³ Cooperative investigations on the insect phases of this disease by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, and the Division of Cereal and Forage Insect Investigations, Bureau of Entomology and Plant Quarantine, were begun in 1933 and concluded with the 1937 season. The results of the last three seasons are given here, in comparison with the first year's results, already published, and include further studies of the occurrence and distribution of bacterial wilt, of the insect vectors of this disease, and of the host range of the wilt organism (*Aplanobacter stewarti* (E. F. Smith) McC.)⁴

BACTERIAL WILT ON CORN

WORK PRIOR TO 1934

A number of investigators during the past 40 years have contributed to the knowledge of seasonal development of bacterial wilt of corn (*Zea mays* L.), but consecutive records of the amount of wilt in any one locality over a long period of years are very limited. Rand and Cash (10) calculated total percentages of wilt for 1918-23 in relation to temperature and rainfall. No consecutive records of the accumulation and spread of wilt from 1923 to the present have been published. Haenseler⁵ has given a general picture of the wilt situation in New Jersey since 1910, and estimated percentages of wilt in different localities are available in Government and State publications. The use of these figures and records in comparison with winter temperatures has led to the conclusion that there is a definite relationship between winter temperatures and wilt development.^{6, 7} Haenseler⁸ reported that a certain time factor or lag period is involved and that after a period of severe wilt a single cold winter may not be sufficient to give the expected disease control, and likewise after a period of little wilt the first warm winter may not be sufficient to give a wilt outbreak the following summer. The desirability of more definite wilt records for the

¹ Received for publication June 28, 1939.

² The writers are greatly indebted to P. N. Annand, formerly in charge of the Division of Cereal and Forage Insect Investigations, for suggestions and aid in the conduct of these studies; to Nancy H. Wheeler and J. W. Scrivener, of the same Division, for invaluable assistance with details of the experimental work; and to H. S. Barber, of the Division of Insect Identification, for continually aiding in identification of flea beetles.

³ Italic numbers in parentheses refer to Literature Cited, p. 686.

⁴ Synonym, *Phytomonas stewarti* (E. F. Smith) Bergey et al.

⁵ HAENSELER, C. M. CORRELATION BETWEEN WINTER TEMPERATURES AND INCIDENCE OF SWEET CORN WILT IN NEW JERSEY. U. S. Bur. Plant Indus., Plant Dis. Rptr. 21: 298-301. 1937. [Mimeographed.]

⁶ HAENSELER, C. M. See footnote 5.

⁷ STEVENS, N. E. STEWART'S DISEASE IN RELATION TO WINTER TEMPERATURE. U. S. Bur. Plant Indus., Plant Dis. Rptr. 18: 141-149. 1934. [Mimeographed.]

1936

Golden Cross Bantam: 3

May 7.....	June 15 10 inches	216	0	0	0	0	0	0	131	22	935
May 21.....	July 13 Tassel	214	7	3.3	2.3	.9	0	0	0	0	0
June 1.....	Aug. 5 Harvested	214	160	74.8	73.8	0	0	0	0	0	0
June 15.....	Aug. 20 10 inches	137	0	0	0	0	0	0	59	14	775
July 1.....	July 13 Beginning to tassel	132	2	1.5	8	8	0	0	0	0	0
July 15.....	Aug. 5 Silk	223	103	78.0	65.2	12.9	0	0	0	0	0
July 1.....	Aug. 10 10 inches	223	1	4	0	4	0	0	0	0	0
July 15.....	Aug. 20 Silk	220	85	40.0	35.0	5.0	0	0	128	22	10
July 1.....	Aug. 25 Harvested	220	110	50.0	49.1	0	0	0	0	0	0
July 15.....	July 13 10 inches	353	0	0	0	0	0	0	132	29	515
July 1.....	Aug. 5 Beginning to tassel	350	33	9.4	8.6	.9	0	0	0	0	0
July 15.....	Aug. 20 Harvested	325	120	36.9	21.5	15.4	0	0	0	0	0
July 1.....	Aug. 5 10 inches	308	17	5.5	4.9	.6	0	0	0	0	0
July 15.....	Aug. 26 Silk	299	293	98.0	83.9	13.4	.7	0	114	18	405
July 1.....	Sept. 21 Harvested	299	299	100.0	0	99.3	0	0	0	0	0
July 15.....	Aug. 11 10 inches	305	2	.7	0	.7	0	0	64	9	425
July 1.....	Aug. 28 Tassel	298	203	68.1	53.7	11.7	2.7	0	0	0	0
July 15.....	Sept. 9 Silk	295	294	99.3	66.2	31.4	1.7	0	0	0	0
July 1.....	Oct. 1 After harvest	294	294	100.0	10.2	86.4	.3	3.1	0	0	0
Total or average.....									678	117	65

1937

Golden Cross Bantam: 1

May 11.....	June 8 6-8 inches	291	22	7.6	.3	5.2	2.1	0	0	0	0
May 17.....	June 21 16 inches	286	29	10.1	.3	6.6	3.1	0	139	27	275
June 1.....	July 16 Silk	286	286	93.0	96.4	3.9	3.7	0	0	0	0
June 14.....	July 17 14-26 inches	237	300	121.7	0	9.3	3.4	0	181	34	969
July 1.....	Aug. 2 Silk	239	232	97.1	8.2	15.9	0	0	0	0	0
July 14.....	Aug. 17 10-12 inches	238	238	100.0	52.6	45.8	1.3	0	161	27	975
July 1.....	Aug. 10 Silk	247	56	22.7	5.1	11.7	4.9	0	0	0	0
July 14.....	Aug. 10 24 inches	244	244	100.0	63.9	32.4	3.7	0	123	23	680
July 1.....	Sept. 4 Silk	279	245	87.8	59.1	20.1	8.6	0	0	0	0
July 17.....	Sept. 16 18-24 inches	279	279	100.0	29.4	66.7	3.9	0	84	13	210
July 1.....	Sept. 16 Silk	229	185	80.8	40.2	29.7	6.6	4.4	5	0	630
Total or average.....			222	96.9	61.6	18.8	8.7	4.8	713	127	739

See footnotes at end of table.

1936

Gill Bros. Improved Golden Bantam
(Oregon seed):

May 7	June 15 Tassel	270	12	4.4	2.9	1.1	0		
	June 29 Harvest	267	15	5.6	4.5	1.1	4		
	July 13 Drying	267	58	21.7	15.4	7.7	28	3	565
	Aug. 3	267	178	66.7	21.0	33.0	6.7		
May 21	July 6 6-12 inches	216	2	9.3	5	5	0		
	July 13 Tassel	216	20	9.3	8.3	9	0		
	Aug. 5 Harvest	216	182	84.3	39.4	40.3	1.4	67	420
June 1	July 6 6-12 inches	263	2	7	0	4	0		
	July 13 Tassel	263	2	7	0	4	0		
	Aug. 5 Silk	262	201	71.3	54.6	16.3	0	103	240
	July 13 4-10 inches	374	0	0	0	0	0		
June 15	Aug. 5 Silk	374	109	29.1	21.4	5.3	2.1	3	
	Aug. 26 Harvest	374	374	100.0	0	70.1	20.1	9.9	940
July 1	Aug. 26 6-12 inches	337	99	29.4	21.7	3.0	3.3	1.5	
	Aug. 5 (Silk to harvest)	331	331	100.0	1.5	76.4	17.2	4.8	140
July 15	Aug. 11 5-10 inches	329	42	12.8	0	9.1	3.0	6	
	Aug. 28 Tassel	329	318	96.7	25.2	35.9	21.6	10.9	
Total or average								328	44 5

1937

Gill Bros. Improved Golden Bantam
(Oregon seed):

May 11	June 8 6-8 inches	258	147	57.0	1.2	21.3	27.9	6.6	
	June 21 12 inches	237	188	79.3	0	34.2	25.3	19.8	40
May 17	July 17 Silk	195	195	100.0	2.1	48.7	32.3	16.9	
	June 21 12 inches	216	199	92.6	0	39.8	30.1	3.7	940
June 1	July 19 Silk	189	189	100.0	1.0	52.3	37.7	9.0	
June 14	July 17 Tassel	268	212	86.2	11.2	44.8	19.1	3.7	785
	Aug. 10 Silk	268	268	100.0	19.2	49.9	13.8	12	
July 1	Aug. 10 10-12 inches	247	246	99.6	9.7	27.1	29.1	23.8	
July 17	Sept. 4 Mature	170	170	100.0	0	1.8	22.9	25.3	
	do 14 inches (mature)	244	244	100.0	1.6	18.4	37.3	42.6	
Total or average								48	6 765

i = a-light infection (small local leaf lesions); b = moderate infection (long leaf lesions extending into midvein); c = heavy infection (abundant leaf lesions, stunted plants with premature tassels); d = plants dead or dying.

* Seed from G. M. Smith.

* Seed from Associated Seed Growers.

* In 1935 increase in numbers was due to tillers (more than 1 plant to a hill).

* Many plants were killed by the fall army worm (*Laphygma frugiperda* S. and A.).

* Poor ears.

study of underlying causes of wilt epiphytotics has been evident for some time. Beginning with 1934 such records have been kept at the Arlington Experiment Farm, Arlington, Va. (near Washington, D. C.) and in several other localities for from 1- to 4-year periods.

ARLINGTON EXPERIMENT FARM, 1934-37

During the four seasons 1934-37, resistant and susceptible varieties of sweet corn were planted at the Arlington farm at intervals of approximately 15 days, beginning about May 1 and continuing through July 15. Golden Cross Bantam was used as the resistant variety, and Golden Bantam inbreds or open-pollinated commercial varieties from sections where the disease does not occur, were used as the susceptible variety. Records were kept of the number of infected plants and of the degree of infection, since the latter differs greatly in resistant and susceptible varieties. Two records of amount of wilt in each planting are given here, as they are representative of the amount of wilt developing in any one planting and variety. The first of these records shows the amount of wilt when the plants were about 10 inches high and the second when they were in tassel and silk. In 1936, because of the late development of the disease, additional records were taken when the ears were ready to harvest and also after harvest. These wilt records are given in table 1, which shows differences in amount of wilt in resistant and susceptible varieties, changes in amount of wilt in each in successive seasons, and differences in early- and late-season development of wilt.

Resistant and susceptible varieties of sweet corn differ in the numbers of lesions developing and in the extent and size of individual lesions. In a comparison of the amount of wilt in the two varieties, 100 percent of infected plants means much more serious damage in susceptible than in resistant varieties. In 1934 all plants of both varieties became infected, but in the resistant variety most of the plants showed only light to moderate infection and only 1 to 8 percent were dead. In the susceptible variety 18 to 20 percent were heavily infected and an additional 19 to 44 percent were dead or dying. Similar differences were apparent in all 4 years. The percentages of heavily infected and dead plants were very low in Golden Cross Bantam and comparatively high in Gill Bros. Improved Golden Bantam. Average percentages of infected plants in all plantings are shown in table 2. Golden Cross Bantam showed much less infection in the early leaf stage, and the lesions were more limited in development; as a result, there was much less reduction in yield.

Although resistant and susceptible varieties differ greatly in degree of infection and reduction in yield, they apparently respond alike to change in season, and striking differences are observed in the records for the four seasons. In 1934, 1935, and 1937 wilt was abundant early in the season, and as the season advanced, more than half of the plants of the susceptible variety became heavily infected or died and few marketable ears were harvested. In the resistant variety most plantings showed some early infection, and in 1934 and 1935 a few heavily infected or dead plants. In 1936 the situation was very different. The well-developed plots of susceptible sweet corn were in marked contrast to the heavily infected plots of the other three seasons. The striking thing about the wilt situation in 1936 was the small number

of infected plants early in the season and the abundant general infection that developed early in August and continued through the remainder of the season. In 1936 infections in both resistant and susceptible varieties were largely confined to the leaves. Since the general infection came too late to interfere with the development of the plants, the percentage of heavily infected or dead plants for the season was low and there was a good crop of marketable ears even on the early susceptible variety.

TABLE 2.—Average percentages of infected plants in all plantings of two varieties of corn

Variety and stage	Plants infected in—			
	1934	1935	1936	1937
Golden Cross Bantam:	Percent	Percent	Percent	Percent
Leaf stage	6.4	19.1	1.3	50.6
Tassel	100.0	54.9	41.4	97.8
Gill Bros. Improved Golden Bantam:				
Leaf stage	59.2	69.7	8.7	79.8
Tassel	100.0	86.5	44.2	99.8

The low wilt records (table 1) for 1936 at the Arlington Farm were even more apparent in other sweet corn plantings. In a series of plots of Top Cross Spanish Gold and Golden Early Market, planted May 22, the results were as shown in table 3.

TABLE 3.—Wilt infection in plots of Top Cross Spanish Gold and Golden Early Market Sweet Corn at the Arlington Experiment Farm, Arlington, Va., 1936

(Planted May 22)

Variety	Plot No.	Date of record	Condition	Rows	Plants					
					Total	In-fected	Showing indicated degree of infection			
							Light	Moderate	Heavy	Dead
Top Cross Spanish Gold	301	June 29	10 inches	No.	No.	Pct.	Pct.	Pct.	Pct.	Pct.
		July 15	Tassel	5	81	.0	.0	.0	.0	.0
		Aug. 6	Silk	5	74	67.6	60.8	6.8	.0	.0
	306	June 29	10 inches	5	63	.0	.0	.0	.0	.0
		July 15	Tassel	5	61	.0	.0	.0	.0	.0
		Aug. 6	Silk	5	60	50.0	43.3	6.7	.0	.0
	312	June 29	10 inches	5	77	.0	.0	.0	.0	.0
		July 15	Tassel	5	74	.0	.0	.0	.0	.0
		Aug. 6	Silk	5	73	21.9	19.2	2.7	.0	.0
	315	June 29	10 inches	5	65	.0	.0	.0	.0	.0
		July 15	Tassel	5	62	.0	.0	.0	.0	.0
		Aug. 6	Silk	5	62	45.2	38.7	6.5	.0	.0
	5 rows 132 feet	June 29	12 inches	5	308	.0	.0	.0	.0	.0
		July 15	Tassel	5	305	3.6	3.0	.7	.0	.0
		Aug. 11	Silk	5	302	88.4	60.9	27.2	.3	.0
	303	June 29	10 inches	5	78	.0	.0	.0	.0	.0
		July 15	Tassel	5	76	7.9	7.9	.0	.0	.0
		Aug. 6	Silk	5	66	48.5	31.8	13.6	1.5	1.5
	305	June 29	10 inches	5	81	.0	.0	.0	.0	.0
		July 15	Tassel	5	78	6.4	5.1	1.3	.0	.0
		Aug. 6	Silk	5	73	83.6	42.5	30.1	8.2	2.7
Golden Early Market	310	June 29	10 inches	5	108	.0	.0	.0	.0	.0
		July 15	Tassel	5	108	2.8	1.9	.0	.0	.9
		Aug. 6	Silk	5	103	29.1	20.4	8.7	.0	.0
	316	June 29	10 inches	5	93	.0	.0	.0	.0	.0
		July 15	Tassel	5	92	2.2	2.2	.0	.0	.0
		Aug. 6	Silk	5	90	28.9	24.4	4.4	.0	.0
	5 rows 132 feet	June 29	10 inches	5	384	1.0	.3	.3	.3	.3
		July 15	Tassel	5	380	11.6	8.4	1.8	.8	.5
		Aug. 11	Silk	5	372	68.5	42.7	20.7	.0	5.1

There were four small plots of five rows each of Top Cross Spanish Gold and Golden Early Market adjacent to the plantings referred to in table 1. In addition, five 132-foot rows of each of these varieties were planted on another section of the Arlington farm, several hundred yards from the small plots and across a narrow roadway from plots of small grain. Top Cross Spanish Gold is more resistant than Golden Early Market. No evidence of wilt infection was found in the small plots of either variety until after the first of July. A few light infections were found in Golden Early Market the middle of July. By the first week in August both varieties showed from about 22 to 88 percent of infected plants. An interesting fact shown in table 3 is that in both varieties wilt developed earlier and was more severe in the five 132-foot rows than in the smaller plots.

Table 4 shows the percentages of infected plants in 10 open-pollinated varieties of sweet corn at the Arlington farm in 1936. Up to the first of July little or no infection appeared on these plants. About half of the plants of each variety were inoculated the first week in July. There were, therefore, two sets of infected plants—those in which the disease was due to natural infection and those in which it was due to inoculation. It is apparent from the data that there was little or no natural infection early in the season. It is also apparent that abundant infection developed in inoculated plants. The season was therefore favorable for each type of infection but the disease failed to develop naturally, evidently because inoculum was wanting. The data in table 4 show also that as the season advanced natural infection became much more abundant, in some cases as abundant as infection on inoculated plants. There were very few corn flea beetles (*Chaetocnema pulicaria* Melsh.) early in the season, but the number increased as the season advanced. The abundance of wilt and the abundance of adults of *C. pulicaria* were apparently closely correlated on the Arlington farm in 1936.

Wilt records at the Arlington farm showed a definite relation of wilt to winter temperatures. In figure 1 are given the mean and mean normal and the minimum and mean minimum temperatures for the winter months December, January, and February, for 1925-37, in Washington, D. C. The mean temperatures for the earlier years are given merely to show the succession of above-normal winters leading up to the unusually mild winters of 1931-32 and 1932-33. The winters of 1933-34 and 1934-35 were similar to each other. In 1933-34 the average winter temperature was slightly below normal but well above 32° F. Minimum temperatures for the 3 months were 8°, 8°, and -6°, respectively, making the average for February 24.6°, or 10° below normal. In 1934-35 the average was slightly above normal, with minimum temperatures of 17°, -2°, and 6°. In 1935-36 the winter was much colder, the average temperature being slightly below 32°. Although the minima of 4°, 0°, and 3° did not quite equal the lowest temperature in each of the preceding years, the average was lower. In 1936-37 the winter was much warmer than normal, the average being above 40° with minima for the 3 months of 14°, 29°, and 20°, respectively, and almost as high an average as for the winter of 1932-33.

TABLE 4.—Percentages of infected plants in open-pollinated varieties of sweet corn at the Arlington Experiment Farm, Arlington, Va., 1936

[Planted May 22]

Sweet corn varieties	Date of record	Condition	Plants							
			Total	Infected		Showing indicated degree of infection				
						a	b	c	d	
			No.	No.	Percent	No.	No.	No.	No.	
Golden Bantam	July 6	About 10 inches	42	1	2.380	0	1	0	0	
	July 14	Tassel	123	16	69.565	7	9	0	0	
	Aug. 5	Harvest	123	0	.00	0	0	0	0	
	July 6	10 inches	123	23	100.00	3	12	8	0	
Early Crosby	July 6	10 inches	23	0	.00	10	1	1	0	
	July 14	Tassel	112	10	83.33	0	0	0	0	
	Aug. 5	Harvest	112	12	100.00	0	2	3	7	
	July 6	About 10 inches	112	9	81.81	8	1	0	0	
Black Mexican	July 6	About 10 inches	25	0	.00	0	0	0	0	
	July 14	Tassel	112	6	50.00	2				
	Aug. 5	Harvest	111	11	100.00	2	9	0	0	
	July 6	About 10 inches	113	9	69.231	9	0	0	0	
Spanish Gold	July 6	About 10 inches	22	0	.00	0	0	0	0	
	July 14	Tassel	111	4	36.36					
	Aug. 5	Mature	111	11	100.00	1	1	5	4	
	July 6	8 inches	111	10	90.90	7	3	0	0	
White Cob Cory	July 6	8 inches	8	0	.00	0	0	0	0	
	July 14	10 inches	13	3	100.00	3	0	0	0	
	Aug. 5	Tassel to silk	25	0	.00	0	0	0	0	
	July 6	10 inches	13	3	100.00	0	0	3	0	
Golden Sunshine	July 6	10 inches	25	5	100.00	5	0	0	0	
	July 14	Tassel	41	0	.00	0	0	0	0	
	Aug. 5	Harvest	121	15	71.428					
	July 6	10 inches	220	0	.00	0	0	0	0	
Whipple's Yellow	July 6	10 inches	121	21	100.00	2	2	9	8	
	July 14	Tassel	20	20	100.00					
	Aug. 5	Harvest	48	2	4.166	0	2	0	0	
	July 6	10 inches	25	19	76.00	0	19	0	0	
Bantam Evergreen	July 6	10 inches	23	1	4.347	0	1	0	0	
	July 14	Tassel	125	23	92.00	1	17	4	1	
	Aug. 5	Harvest	23	11	47.826	10	1	0	0	
	July 6	10 inches	26	0	.00	0	0	0	0	
Stowell Evergreen	July 6	10 inches	112	10	83.33	0	10	0	0	
	July 14	Tassel	114	1	7.142	0	1	0	0	
	Aug. 5	Silk	112	11	91.666	2	7	2	0	
	July 6	About 10 inches	114	10	71.429	10	0	0	0	
Country Gentleman	July 6	About 20 inches	41	0	.00	0	0	0	0	
	July 14	Tassel and silk	17	11	64.705	11	0	0	0	
	Aug. 5	16 inches	16	0	.00	0	0	0	0	
	July 6	10 inches	17	12	70.588	2	10	0	0	
	Aug. 5	16 inches	16	3	18.75	3	0	0	0	
	July 6	10 inches	31	0	.00	0	0	0	0	
	Aug. 5	Silk	17	5	29.411	5	0	0	0	
	July 14	16 inches	14	0	.00	0	0	0	0	
	Aug. 5	Silk	16	11	68.75	6	5	0	0	
			13	2	15.385	2	0	0	0	

¹ Plants inoculated July 6 to July 9 with *Aplanobacter stewartii*, 36-15-40.² Not inoculated.

Unfortunately, there are no wilt records for the epiphytotic year of 1933 for comparison with succeeding years and therefore no way of demonstrating that average percentages of infection were lower in 1934, although, from field observations, this was undoubtedly the case. The average winter temperature for 1934-35 was slightly above that for the preceding year, the averages for December and February being slightly above and for January slightly below normal. In Golden Cross Bantam early infection in the first planting was less in 1935 than in 1934, but the records for the susceptible variety show more early infection. The average percentages for the 2 years indicate less wilt in 1935 than in 1934. But the very decided decrease in wilt

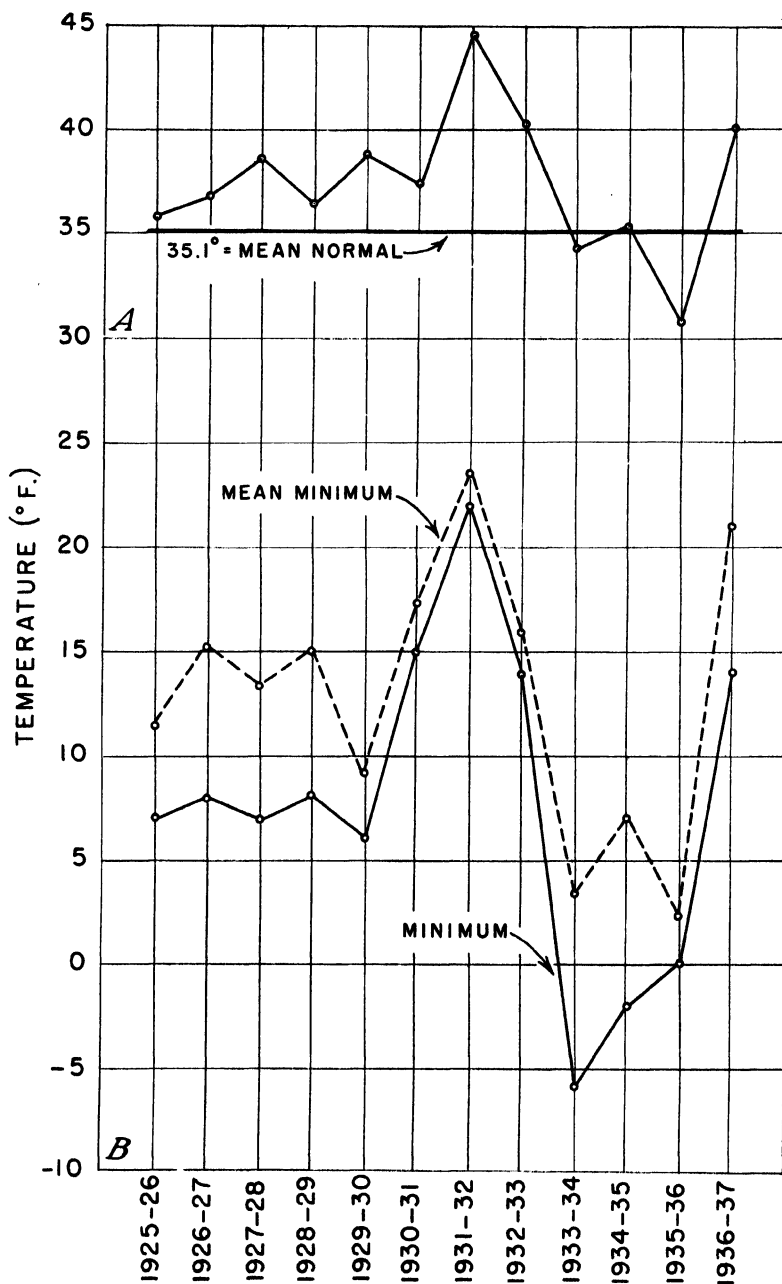


FIGURE 1.—Temperatures for December, January, and February, 1925-37, Washington, D. C.: A, Mean temperatures; B, minimum and mean minimum temperatures.

came in 1936, following the consistently low temperatures of the winter months; and wilt was abundant again early and throughout the season in 1937, following the warm winter of 1936-37. Wilt at the Arlington farm has followed closely these changes in winter temperature.

Because of the small size of the corn flea beetle, its extreme activeness, and other habits that are influenced directly by weather conditions and other factors, no practicable method of determining exact populations of this insect has been developed. However, field collections and observations on number of beetles and number of feeding injuries on corn leaves at the Arlington farm during 1934, 1935, and 1937 have shown that these beetles were much more abundant on young corn early in the season of these years than in 1936, when it was difficult at times to collect enough beetles for experimental purposes. At the same time there was little spread of the disease from the primary infections early in 1936. As the season advanced the adults of *Chaetocnema pulicaria* increased in number until August when they were fairly abundant on corn.

In 1937, corn flea beetles were again abundant early in the season. Factors not yet well understood are undoubtedly important in influencing winter survival of this insect. With few beetles to spread the primary infections in the spring and early summer, early infection appears to be reduced.

With a warm winter such as that of 1936-37 at Washington, D. C., following the late-season increase in number of beetles and general late-season infection on the corn plants in 1936, any retarding effect of one cold winter upon the early development of wilt the second season, which Haenseler⁸ has called a "lag" period, would not be apparent. In colder sections, where beetles may be less abundant and infection of corn plants less general, the retarding effect of a single cold winter probably would be more apparent.

In the plantings of Top Cross Spanish Gold and Golden Early Market it was noted that, in both varieties, wilt developed earlier and was more severe in the five long rows than in the smaller plots. *Chaetocnema pulicaria* appeared to be more abundant on these long rows than on other corn plots on the Arlington farm and probably moved onto these corn rows early in July from the small-grain plots located across a narrow roadway.

OTHER LOCALITIES, 1935-37⁹

Records have been made of the development of bacterial wilt in plantings at Ithaca, N. Y., in 1935, at Yonkers, N. Y., in 1935, 1936, and 1937, and at La Fayette, Ind., Toledo, Ohio, Geneva, N. Y., and Hempstead, Long Island, N. Y., in 1936 and 1937, and from these plantings field collections of possible insect vectors were made during the growing season. The wilt records from these stations are given in table 5.

⁸ See footnote 5.

⁹ The writers are indebted to the following persons for aid in this work: To G. M. Smith, of the Bureau of Plant Industry, for taking records on the disease at La Fayette, Ind., and at Toledo, Ohio; to E. V. Walter, of the Bureau of Entomology and Plant Quarantine, for collections of insects from Indiana; to Ralph Mathes, of the same Bureau, for the collections of insects from Toledo; to W. A. Baker, of the same Bureau for maintenance of the sweet-corn plots at Toledo; to B. L. Richards, of the Nassau County Farm Bureau, for assistance with the field plots and for making most of the collections of insects on Long Island; and to H. G. Walker and L. D. Anderson, of the Virginia Truck Experiment Station, for most of the insect collections obtained from Norfolk, Va.

TABLE 5.—Percentages of infected plants in plantings of resistant and susceptible sweet corn varieties at various localities

GENEVA, N. Y.

Variety	Date planted	Date of record	Condition on date of record	Rows	Plants							
					Total	Infected	Showing indicated degree of infection					
							Light	Moderate	Heavy	Dead		
Golden Cross Bantam	1936			No.	No.	No.	Pct.	Pct.	Pct.	Pct.		
	May 15	June 19	10 inches	2	47	0	0.0					
		Aug. 14	Silk	2	41	0	0					
	June 1	do	do	2	40	0	0					
	June 15	do	Tassel	1	66	0	0					
	1937											
	June 26	Aug. 28	Silk	1	85	6	7.1	3.5	3.5	0.0	0.0	
	July 15	do	18 inches	1	74	1	1.4	1.4	0	0	0	
	Aug. 2	do	6 inches	1	121	0	0	0	0	0	0	
	Gill Bros. Improved Golden Bantam	1936										
May 15		June 19	12 inches	2	58	0	0					
		Aug. 14	Harvest	2	58	0	0					
June 1		do	Silk	2	44	0	0					
June 15		do	Tassel	1	70	0	0					
1937												
June 26		Aug. 28	Silk	1	77	8	10.4	10.4	0	0	0	
July 15		do	Tassel	1	110	12	10.9	6.4	4.5	0	0	
Aug. 2		do	6 inches	1	130	0	0	0	0	0	0	

ITHACA, N. Y.

Golden Cross Bantam	1935													
	May 26	June 29	10 inches	3	214	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Aug. 15	Silk	3	252	5	2.0	.8	1.2	0	0	0	0	0
	June 6	June 29	6 inches	3	250	0	0	0	0	0	0	0	0	0
		Aug. 15	Silk	3	254	3	1.2	1.2	0	0	0	0	0	0
	June 20	do.	Tassel	3	238	1	.4	.4	0	0	0	0	0	0
Gill Bros. Improved Golden Bantam	June 28	do.	3 feet	3	246	0	0	0	0	0	0	0	0	0
	May 26	June 29	8 inches	3	136	0	0	0	0	0	0	0	0	0
		Aug. 15	Silk	3	198	6	3.0	2.0	1.0	0	0	0	0	0
	June 6	June 29	4 inches	3	0	0	0	0	0	0	0	0	0	0
		Aug. 15	Silk	3	192	7	3.6	1.0	2.6	0	0	0	0	0
	June 20	do.	Tassel	3	112	2	1.8	.9	0	0	0	0	0	0
Golden Bantam	June 28	do.	do.	3	120	3	.5	2.0	2.5	0	0	0	0	0
	(1)	June 29	do.	3	100	0	0	0	0	0	0	0	0	0

YONKERS, N. Y.

Golden Cross Bantam	1935													
	May 15	June 28	10 inches	3	131	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Aug. 19	Harvest	3	277	0	0	0	0	0	0	0	0	0
	May 31	June 28	8 inches	3	233	1	.4	0	0	0	0	0	0	0
		Aug. 19	Silk	3	290	0	0	0	0	0	0	0	0	0
	June 15	June 28	4 inches	3	0	0	0	0	0	0	0	0	0	0
Gill Bros. Improved Golden Bantam	June 1	Aug. 19	Silk	3	172	2	1.2	1.2	0	0	0	0	0	0
		do.	Tassel	3	314	0	0	0	0	0	0	0	0	0
	May 15	June 28	10 inches	3	136	2	1.5	0	.7	7	0	0	0	0
		Aug. 19	Tassel	3	191	17	8.9	1.6	4.2	0	0	0	0	0
	May 31	June 28	6 inches	3	237	3	1.2	1.2	0	0	0	0	0	0
		Aug. 19	Roasting ear	3	257	11	4.3	1.2	.4	0	0	0	0	0
Golden Bantam	June 15	June 28	Silk	3	141	1	.7	0	0	0	0	0	0	0
		Aug. 19	Tassel	3	240	2	.8	.4	0	0	0	0	0	0
	(1)	June 28	20 inches	6	616	11	1.8	.3	.3	.5	.6	.6	.6	.6
		Aug. 19	14 inches	3	333	30	9.0	0	.9	3.3	4.4	4.4	4.4	4.4
	1936													
	(1)	June 17	10 inches	6	700	0	0	0	0	0	0	0	0	0
Golden Bantam		Aug. 13	Silk	6	700	26	3.7	3.4	0	0	0	0	0	0
	1937													
	May 18	June 25	12 inches	1	246	12	4.9	.4	2.4	1.2	.8	.8	.8	.8
	June 2	Aug. 27	Harvest	1	113	112	99.1	0	67.3	16.8	15.0	15.0	15.0	15.0
Golden Bantam	June 17	do.	Silk	1	166	165	99.4	5.4	180.1	9.0	4.8	4.8	4.8	4.8

1 Planted in home garden but date not known.

TABLE 5.—Percentages of infected plants in plantings of resistant and susceptible sweet corn varieties at various localities—Continued

HEMPSTEAD, LONG ISLAND, N. Y.

Variety	Date planted	Date of record	Condition on date of record	Rows	Plants							
					Total	Infected			Showing indicated degree of infection			
									Light	Moderate	Heavy	Dead
	1936			No.	No.	No.	Pct.	Pct.	Pct.	Pct.	Pct.	
Golden Cross Bantam	May 17	June 17	10 inches	1	71	0	0.0	0.0	0.0	0.0	0.0	
		July 9	Tassel	1	71	2	2.8	0	0	2.8	0	
		Aug. 13	Silk	1	68	68	100.0	79.4	16.2	4.4	0	
	June 1	July 9	Tassel	1	71	0	0	0	0	0	0	
		Aug. 13	Harvest	1	73	72	98.6	86.3	6.8	5.5	0	
	June 16	do	Tassel	1	75	67	89.3	68.0	16.0	5.3	0	
		1937										
Gill Bros. Improved Golden Bantam	May 15	June 25	10 inches	1	97	7	7.2	0	1.0	6.2	0	
	June 1	do	6 inches	1	101	0	0	0	0	0	0	
	June 15	do	3 inches	1	91	0	0	0	0	0	0	
		Aug. 27	Mature	1	91	91	100.0	72.5	27.5	0	0	
		1936										
	May 13	June 17	12 inches	1	82	2	2.4	0	1.2	1.2	0	
		July 9	Tassel	1	79	13	16.5	2.5	2.5	8.9	2.5	
	Aug. 13	Mature	1	79	60	87.3	26.6	36.7	3.8	20.3		
	June 1	July 9	Tassel	1	80	10	12.5	1.3	5.0	5.0	1.3	
		Aug. 13	Harvest	1	74	74	100.0	39.2	36.5	17.6	6.8	
	June 16	do	Silk	1	73	71	97.3	63.0	19.2	11.0	4.1	
		1937										
	May 15	June 25	8 inches	1	104	9	8.7	0	0	3.8	4.8	
	June 1	do	5 inches	1	92	1	1.1	0	1.1	0	0	
	July 1	Aug. 27	Mature	1	83	83	100.0	6.0	32.5	53.0	8.4	

TOLEDO, OHIO²

Golden Cross Bantam	1936											
	May 15	Aug. 1		1	225	3	1.3					
	June 1	do		1	246	1	.4					
	June 15	do		1	316	0	0.0					
	1937											
	May 15	June 25		1	187	0	0.0	0.0	0.0	0.0	0.0	0.0
		July 20		1	187	0	0.0	0.0	0.0	0.0	0.0	0.0
		Aug. 13		1	187	0	0.0	0.0	0.0	0.0	0.0	0.0
	June 1	June 25		1	220	0	0.0	0.0	0.0	0.0	0.0	0.0
		July 20		1	220	0	0.0	0.0	0.0	0.0	0.0	0.0
Gill Bros. Improved Golden Bantam	June 15	July 20		1	215	0	0.0	0.0	0.0	0.0	0.0	0.0
		Aug. 13		1	215	2	.9	.9	0.0	0.0	0.0	0.0
		Sept. 4		1	215	31	14.4	11.6	0.0	1.9	.9	0.0
	July 1	July 20		1	183	0	0.0	0.0	0.0	0.0	0.0	0.0
		Aug. 13		1	183	4	2.2	.5	0.0	.5	1.1	0.0
		Sept. 4		1	183	13	7.1	4.4	0.0	2.7	0.0	0.0
	1936											
	May 15	Aug. 1		1	266	1	.4					
	June 1	do		1	299	2	.7					
	June 15	do		1	311	0	0.0					
Gill Bros. Improved Golden Bantam	1937											
	May 15	June 25		1	148	0	0.0	0.0	0.0	0.0	0.0	0.0
		July 20		1	148	1	.7	0.0	0.0	0.0	0.7	0.0
		Aug. 13		1	148	12	8.1	.7	0.0	2.0	5.4	0.0
	June 1	June 25		1	168	0	0.0	0.0	0.0	0.0	0.0	0.0
		July 20		1	168	0	0.0	0.0	0.0	0.0	0.0	0.0
		Aug. 13		1	168	2	1.2	0.0	0.0	.6	.6	0.0
	June 15	July 20		1	177	0	0.0	0.0	0.0	0.0	0.0	0.0
		Aug. 13		1	177	5	2.8	2.8	0.0	0.0	0.0	0.0
		Sept. 4		1	177	177	100.0	65.5	0.0	16.9	17.5	0.0
Gill Bros. Improved Golden Bantam	July 1	July 20		1	193	0	0.0	0.0	0.0	0.0	0.0	0.0
		Aug. 13		1	193	19	9.8	.5	0.0	2.1	7.3	0.0
		Sept. 4		1	193	159	82.4	38.9	0.0	30.1	13.5	0.0

² Notes taken by Glenn M. Smith.

TABLE 5.—Percentages of infected plants in plantings of resistant and susceptible sweet corn varieties at various localities—Continued

LA FAYETTE, IND.²

Variety	Date planted	Date of record	Condition on date of record	Rows	Plants							
					Total	Infected		Showing indicated degree of infection				
						No.	Pct.	Light	Moderate	Heavy	Dead	
				No.	No.	No.	Pct.	Pct.	Pct.	Pct.	Pct.	
Golden Cross Bantam	1936											
	May 15	July 31		1	201	0	0.0					
	June 1	do		1	215	0	.0					
	June 15	do		1	190	0	.0					
	1937											
	May 15	June 26		1	217	0	.0	0.0	0.0	0.0	0.0	
		July 21		1	217	0	.0	.0	.0	.0	.0	
		Aug. 14		1	217	0	.0	.0	.0	.0	.0	
	June 1	June 26		1	267	0	.0	.0	.0	.0	.0	
		July 21		1	267	0	.0	.0	.0	.0	.0	
		Aug. 14		1	267	0	.0	.0	.0	.0	.0	
	June 15	July 21		1	241	0	.0	.0	.0	.0	.0	
		Sept. 5		1	241	0	.0	.0	.0	.0	.0	
	June 30	July 21		1	203	0	.0	.0	.0	.0	.0	
		Aug. 14		1	203	5	2.5	1.5	.0	.5	.5	
		Sept. 5		1	203	10	4.9	2.5	.0	2.0	.5	
Gill Bros. Improved Golden Bantam	1936											
	May 15	July 31			195	0	.0					
	June 1	do			190	0	.0					
	June 15	do			210	0	.0					
	1937											
	May 15	June 26		1	179	0	.0	.0	.0	.0	.0	
		July 21		1	179	0	.0	.0	.0	.0	.0	
		Aug. 14		1	179	0	.0	.0	.0	.0	.0	
	June 1	June 26		1	223	0	.0	.0	.0	.0	.0	
		July 21		1	223	0	.0	.0	.0	.0	.0	
		Aug. 14		1	223	0	.0	.0	.0	.0	.0	
	June 15	July 21		1	211	0	.0	.0	.0	.0	.0	
		Aug. 14		1	211	0	.0	.0	.0	.0	.0	
	June 30	Sept. 5		1	211	117	55.5	35.5	.0	11.4	8.5	
		July 21		1	171	0	.0	.0	.0	.0	.0	
		Aug. 14		1	171	10	5.8	.6	.0	.6	4.7	
		Sept. 5		1	171	163	95.3	48.5	.0	35.1	11.7	

² Notes taken by Glenn M. Smith.

At Geneva, N. Y., the northernmost station, not a single wilt lesion was found in 1936 in either variety up to August 14, when the plants were tasseling and the last record was taken. Also, no wilt lesions were found in the station variety plots. In 1937, 7 percent of the plants of the resistant variety were infected and almost 11 percent of the susceptible variety.

At Ithaca, a little farther south, records are available only for 1935, when leaf lesions occurred on 2 percent or less of the resistant variety and on not more than 4 percent of the plants of the susceptible variety.

At Yonkers, which is in the southeastern part of New York, still farther south than Ithaca, field plots of Golden Cross and Gill Bros. Improved Golden Bantam were planted at Boyce Thompson Institute only in 1935. In 1936 and 1937 wilt records from the same garden were made from plantings of commercial Golden Bantam. In 1935 only 1 percent of wilted plants occurred in the resistant Golden Cross and 9 percent in the susceptible Gill Bros. Improved Golden Bantam. But infection apparently took place early enough to kill a large part

of the infected plants. In 1936 no wilt was found on June 17 on 700 Golden Bantam plants. On August 13, when the plants were in silk, 26, or 4 percent, were infected. Two of these plants showed heavy infection, and 24 light or recent infection. In 1937 wilt was much more abundant. Five percent of the plants were infected early in their growth, and 99 percent when mature or in silk, 17 percent being heavily infected and 15 percent dead.

Wilt conditions on Long Island in 1936 were similar to those at the Arlington farm in seasonal development and amount of wilt. There was little wilt early in the season but abundant wilt by the middle of August and relatively few plants heavily infected or dead. Wilt records for 1937 are incomplete, but they show more early infection than in 1936.

At Toledo, Ohio, only late records are available for 1936, and these show less than 2 percent of infected plants. In 1937 no wilt was found on Golden Cross Bantam before the middle of August and only traces in Gill Bros. Improved Golden Bantam, but by the first of September, wilt had become general on the latter variety.

Although no wilt was found on the plots at La Fayette, Ind., in 1936, 0.2 and 0.25 percent of infected plants in two inbred lines, June 29, and 0.066 percent of infected plants in a field of 30,000 plants of susceptible varieties, were reported for the same locality in 1936.¹⁰ In the same publication another observer reported 1 to 2 percent of infected plants, June 10, in open-pollinated Golden Bantam, about 25 miles south of La Fayette, and 10 percent, about 90 miles south, on June 13. These figures are cited to show that wilt was present in small amounts in the vicinity of La Fayette in 1936, although it did not appear in the small experimental plantings. No wilt was observed in La Fayette early in 1937, but by September over 50 and 90 percent of two plantings were infected and nearly half of the plants were heavily infected or dead.

Mean winter temperatures and minimum temperatures for 1925-37 are given in figure 2 for the above mentioned stations or for the nearest weather stations.

Mean temperatures for Geneva, N. Y., show most winter temperatures well below 32° F. with minimum temperatures usually well below 0° and as low as -31°. At Geneva no wilt or only traces occurred in most years. In 1936, when mean winter temperatures were below 32° and when no wilt was found, no specimens of *Chaetocnema pulicaria* were collected in three attempts, weather conditions at this time not being the most favorable for collecting this species. However, evidence of feeding by adult *C. pulicaria* was observed at Geneva, August 25, showing that there were a few beetles in that locality late in the season. In 1937, when mean winter temperatures were well above 32° and when as many as 10 percent of the plants were infected with wilt, 57 specimens of *C. pulicaria* were collected August 18, and 10 of these, or 17.5 percent, were found to be infested with *Aplanobacter stewarti*.

At Toledo, Ohio, mean winter temperatures were somewhat higher than at Geneva, but still were well below 32° F., with minimum temperatures usually well below 0°, in most years. At Toledo, in 1936, only an occasional specimen of *Chaetocnema pulicaria* was collected up

¹⁰ UNITED STATES BUREAU OF PLANT INDUSTRY. BACTERIAL WILT OF CORN IN 1936. U. S. Bur. Plant Indus., Plant. Dis. Rptr. 20: 252. 1936. [Minicographed.]

to the last of July. From that time on, several hundred beetles were collected and sent in for isolation tests, but not an infested beetle was

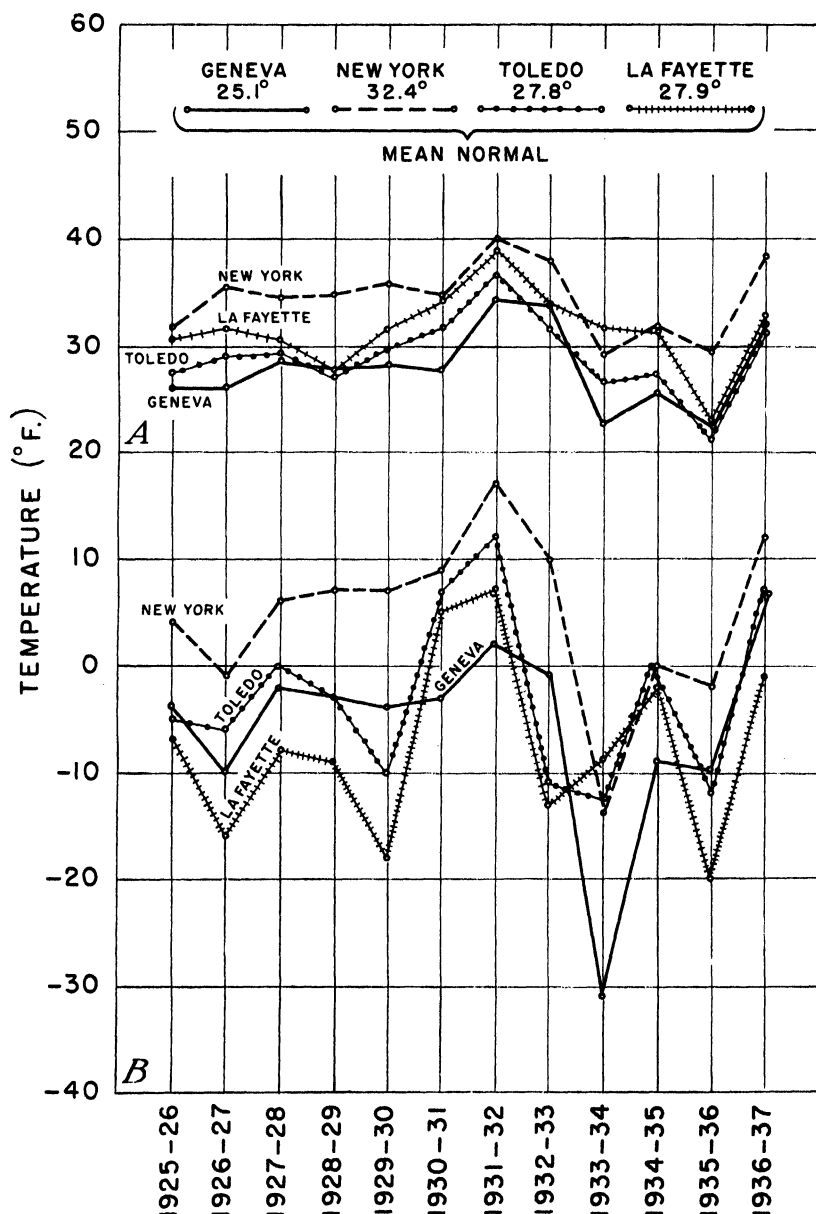


FIGURE 2.—Temperatures for December, January, and February, 1925-37, at Geneva, N. Y., New York, N. Y., Toledo, Ohio, and La Fayette, Ind.: A, Mean temperatures; B, minimum temperatures.

found. It will be recalled that less than 2 percent of infected plants were recorded for Toledo in 1936. In 1936-37 at Toledo, mean winter

temperatures were well above normal and just a little below 32°, and the minimum for all three winter months was 7°. Larger numbers of *C. pulicaria* were collected in June and July as well as later in the season. Only about 1 percent were infested in June and July, but 50 percent were infested the middle of August.

At La Fayette, mean winter temperatures were near 32° F., but minimum temperatures were often well below 0°. In 1935-36 temperatures went much lower, with an average below 24° and a minimum of -20°. No wilt was found in the field plots in July of 1936. A few beetles were collected the middle of the season (74 on June 23) but none was infested. Fairly large numbers were collected as the season advanced, and the percentage infested also increased. In 1936-37 winter temperatures were well above normal and a little above 32°, with a minimum of -1°. No wilt was recorded during June and July of 1937, but in September it became general on susceptible sweet corn. Fairly large numbers of *Chaetocnema pulicaria* were collected throughout the season. Percentages infested were low early in the season and increased as the season advanced.

On Long Island and in Yonkers, N. Y., where mean winter temperatures and minimum temperatures are higher than at Geneva, wilt is more abundant.

It is apparent from these data that the numbers of wilt-infected plants are closely associated with winter temperatures. In sections where mean winter temperatures are well below 32° F., fewer corn flea beetles apparently survive than where mean winter temperatures are well above 32°. It is also evident at the Arlington farm that the beetles may increase greatly in number during a growing season and so probably become a source of infection for the following year if enough of them survive until the corn crop develops. Factors not clearly understood may in some seasons prevent midsummer increase in the severity of wilt, even in the presence of an abundance of beetles.

INSECT VECTORS OF *APLANOBACTER STEWARTI*

The work on insect vectors was continued along lines previously reported for 1934 (9), and methods already described were followed. For the most part, insects that feed on corn under field conditions were collected from various localities, disinfested externally, and crushed in broth from which dilution plates were poured to determine which species and what proportion of the individuals of each species were carrying *Aplanobacter stewarti* internally. Most of the isolations from *Chaetocnema pulicaria* were made from individual insects. Isolations from other species were made from broth suspensions of from 1 to as many as 123 specimens. During 1934, isolations for *A. stewarti* were made from 7,331 insects representing 37 species belonging to 32 genera; in 1935, from 9,390 insects representing 57 species belonging to 48 genera; in 1936, from 5,696 insects representing 60 species belonging to 49 genera; and in 1937, from 6,352 insects representing 29 species belonging to 24 genera, making for the 4 years a total of 28,769 insects from 94 species belonging to 76 genera. Twenty-seven additional species not previously used were tested for the wilt organism in 1935, 23 in 1936, and 7 in 1937. In many instances, the number of isolations from a single species was small because few individuals belonging to the species were collected from corn. In 1937 isolations were made from a few species that were

collected only from hibernating quarters near infected cornfields of the previous year, because of the possibility of their harboring the organism over winter. The results of the isolations made during the period 1934-37 are summarized in table 6, in which 94 species belonging to 76 genera, involving a total of 28,769 specimens, are tabulated. *A. stewarti* was obtained from 27 percent of the *C. pulicaria* in 1934, from 22 percent in 1935, from 20 percent in 1936, and from 30 percent in 1937; and from 7 percent of the *C. denticulata* in 1934, from 4 percent in 1935, from 2 percent in 1936, and from 4 percent in 1937.

TABLE 6.—Summary of insects¹ collected in the field in 1934-37 and tested for *Aplanobacter stewarti*

INSECTS FROM WHICH *A. STEWARTI* WAS ISOLATED

Order and scientific name ²	Common name	Year	Total specimens	Localities	Species of hosts	Isolations made	Isolations tested by inoculation on corn	Isolations of <i>A. stewarti</i>
			No.	No.	Approximate No.	No.	No.	Pct.
Coleoptera:								
<i>Chaetocnema confinis</i> Crotch. ³	Sweetpotato flea beetle (a. n. o.) ⁴	1934	173	9	6	32	7	6.3
		1935	706	30	11	111	13	1.8
		1936	387	11	6	73	9	.0
		1937	23	4	2	22	0	.0
<i>Chaetocnema denticulata</i> (Ill.). ³	Toothed flea beetle	1934	905	16	9	366	89	7.1
		1935	597	28	9	112	17	4.3
		1936	95	11	8	82	7	2.4
		1937	409	15	8	289	35	4.2
<i>Chaetocnema pulicaria</i> Melsh. ³	Corn flea beetle (a. n. o.).	1934	4,790	29	12	3,360	1,330	27.3
		1935	5,588	45	10	5,404	1,420	21.8
		1936	3,809	20	5	3,809	881	19.5
		1937	4,426	26	8	4,426	1,470	29.7
<i>Diabrotica duodecimpunctata</i> (Fab.). ³	Spotted cucumber beetle (a. n. o.).	1934	178	3	5	20	7	.0
		1935	322	24	10	54	6	5.6
		1936	127	4	5	33	4	.0
		1937	11	2	2	7	1	.0
<i>Disonycha glabrata</i> (Fab.).	Flea beetle	1934	2	1	1	1	1	100.0
<i>Epitrix cucumeris</i> (Harr.). ³	Potato flea beetle (a. n. o.).	1934	365	13	6	49	5	.0
		1935	332	18	8	27	5	3.7
		1936	255	10	8	37	8	5.4
		1937	198	6	3	8	0	.0
<i>Phalacrus</i> sp.	Smut beetle (a. n. o.)	1936	90	8	6	22	2	.0
		1937	31	4	12	1	1	8.3
<i>Stilbus apicalis</i> (Melsh.). ³	Shining flower beetle	1934	7	1	1	2	1	50.0
		1935	138	8	7	16	4	6.3
		1936	1	1	1	1	0	.0
		1937	2	1	1	1	1	.0
<i>Stilbus viduus</i> Casey. ³	do.	1934	22	1	1	3	1	.0
		1935	142	16	7	24	4	4.2
		1936	23	4	5	6	4	.0
		1937	38	6	5	12	0	.0
Hemiptera:								
<i>Nabis ferus</i> (L.). ³	Nabid bug	1934	1	1	1	1	1	.0
		1935	1	1	1	1	0	.0
		1936	22	2	3	7	2	14.3
		1937	4	2	2	2	1	.0
Homoptera:								
<i>Illinoia solanifolii</i> (Ashm.). ³	Potato aphid (a. n. o.)	1934	155	2	1	55	5	1.8
		1935	260	2	1	4	0	.0
<i>Stirellus bicolor</i> (Van D.). ³	Leafhopper	1934	4	1	1	1	1	100.0
		1935	3	1	1	2	0	.0
<i>Thamnotettix nigrifrons</i> (Forbes). ³	Black-faced leafhopper	1934	132	1	1	5	2	20.0
		1935	88	2	1	7	2	.0
		1936	1	1	1	1	0	.0
Thysanoptera:								
<i>Anaphothrips obscurus</i> (Müll.).	Grass thrips	1934	66	2	1	9	1	.0
		1935	70	3	1	4	0	.0
		1936	467	1	1	10	3	10.0

¹ Listed alphabetically according to orders; under each order, alphabetically by genera.

² The assistance of various taxonomic specialists in the Bureau of Entomology and Plant Quarantine is gratefully acknowledged.

³ Also tested for *Aplanobacter stewarti* after feeding for several days on infected corn in cages, but data not included in this summary.

TABLE 6.—*Summary of insects¹ collected in the field in 1934-37 and tested for Aplanobacter stewarti—Continued*
INSECTS FROM WHICH A. STEWARTI WAS NOT ISOLATED

Order and scientific name	Common name	Years tested	Insects tested
<i>Coleoptera:</i>			<i>Number</i>
<i>Aeolus amabilis</i> Lec.		1937	108
<i>Brachytarsus sticticus</i> Boh.		1935	5
<i>Ceratomegilla fuscilabrus</i> (Muls.) ³	Spotted ladybird	1935-37	29
<i>Cerotoma trifurcata</i> (Forst.)	Bean leaf beetle	1935	1
<i>Charactenema minuta</i> Melsh.	Flea beetle	1935	11
<i>Chalcidognathus pennsylvanicus</i> (DeG.)	Soldier bug	1935, 1947	13
<i>Collops quadrimaculatus</i> (Fab.)	Four-spotted beetle	1934-37	10
<i>Crepidodera atriventris</i> Melsh.	Acalypha flea beetle	1936	17
<i>Diabrotica longicornis</i> (Say)	Corn rootworm (a. n. o.) ⁴	1935-36	3
<i>Diabrotica vittata</i> (Fab.)	Striped cucumber beetle (a. n. o.)	1935, 1937	7
<i>Dibolia borealis</i> Chev.	Leaf beetle	1934, 1935	51
<i>Dibolia</i> sp.	do	1936	2
<i>Disonycha xanthomelaena</i> (Daln.) ³	Spinach flea beetle	1934-37	14
<i>Epicaula pennsylvanica</i> (DeG.)	Black blister beetle (a. n. o.)	1936	4
<i>Epilachna varivestis</i> Mul.	Mexican bean beetle (a. n. o.)	1934-37	12
<i>Epitrix parvula</i> (Fab.) ³	Tobacco flea beetle (a. n. o.)	1934-36	61
<i>Epitrix</i> sp.	Flea beetle	1936	3
<i>Euphoria inda</i> (L.)	Humble flower beetle (a. n. o.)	1936	1
<i>Glyptochilus fasciatus</i> (Oliv.)		1936	2
<i>Hippodamia convergens</i> Guer. ³	Convergent ladybeetle (a. n. o.)	1935, 1937	7
<i>Hippodamia parenthesis</i> (Say)	Ladybeetle	1936	1
<i>Hypera nigrostris</i> (Fab.)	Lesser clover leaf weevil (a. n. o.)	1935	7
<i>Hypera punctata</i> (Fab.)	Clover leaf weevil (a. n. o.)	1934, 1936	4
<i>Lacon rectangularis</i> (Say)		1937	8
<i>Languria mozdari</i> Latr.	Clover stem borer	1937	11
<i>Leptinotarsa decemlineata</i> (Say)	Colorado potato beetle (a. n. o.)	1936	6
<i>Lixus concavus</i> Say	Rhubarb curculio (a. n. o.)	1936	1
<i>Macrodaetylus subspinosus</i> Fab.	Rose chafer (a. n. o.)	1935	13
<i>Mantura floridana</i> Crotch		1935, 1937	8
<i>Melanophthalma distinguenda</i> Com.		1935-37	48
<i>Meligethes sacrus</i> LeC.		1935	31
<i>Nodonota puncticollis</i> (Say)		1935	4
<i>Notorus calceatus</i> Horn	Rose leaf beetle (a. n. o.)	1937	1
<i>Phalacrox politus</i> Melsh.	Smut beetle (a. n. o.)	1934-36	34
<i>Phyllotreta vittata</i> (Fab.)	Striped flea beetle (a. n. o.)	1934-36	23
<i>Popillia japonica</i> Newm.	Japanese beetle (a. n. o.)	1937	31
<i>Psyllodes</i> sp.	Flea beetle	1935-36	33
<i>Scymnus</i> sp.	Ladybeetle	1936	11
<i>Sitona cylindricollis</i> (Fab.)	Curculio	1936	4
<i>Sitona hispidula</i> (Fab.)	Clover root curculio (a. n. o.)	1936	1
<i>Stilbus</i> sp.	Shining flower beetle	1936-37	27
<i>Systena blanda</i> (Melsh.)	Pale-striped flea beetle (a. n. o.)	1934-36	8
<i>Systena hudsonia</i> (Forst.)	Flea beetle	1935	1
<i>Systena lacinata</i> Auct.	do	1935-36	24
<i>Tetraptes tetraophthalmus</i> Forst.	Red milkweed beetle (a. n. o.)	1935-36	2
<i>Triachus atomus</i> (Suffr.)		1936	2
<i>Collembola:</i>			
<i>Sminthurus</i> sp.	Springtail	1934	8
<i>Hemiptera:</i>			
<i>Adelphocoris rapidus</i> (Say) ³	Rapid plant bug (a. n. o.)	1934-36	4
<i>Allocoris pulicaria</i> (Germ.)		1936-37	3
<i>Anasa armigera</i> (Say)		1937	1
<i>Bliassus leucopterus</i> (Say)	Chinch bug (a. n. o.)	1936-37	989
<i>Euschistus variolarius</i> (P. de B.)	Stinkbug	1936	8
<i>Geocoris punctipes</i> (Say) ³	Plant bug	1934-35	8
<i>Halticus citri</i> (Ashm.) ³	Garden flea hopper (a. n. o.)	1936	21
<i>Lygus pratensis oblineatus</i> (Say) ³	Tarnished plant bug (a. n. o.)	1934-37	191
<i>Ortus insidiosus</i> (Say) ³	Insidious flower bug	1934-36	517
<i>Trigonotylus ruficornis</i> (Geoffr.)		1935-36	29
<i>Homoptera:</i>			
<i>Aceratagallia sanguinolenta</i> (Prov.)	Clover leafhopper	1934-36	7
<i>Agallia constricta</i> Van D. ³	Leafhopper	1934-36	73
<i>Aphis maidis</i> Fitch ³	Corn leaf aphid (a. n. o.)	1936	19
<i>Balclutha</i> sp.	Leafhopper	1935	5
<i>Chlorotettix viridius</i> Van D.	do	1935	1
<i>Delphacodes</i> sp.		1935	1
<i>Deltocephalus flavicostus</i> Stal.	Leafhopper	1934	1
<i>Draculacephala mollipes</i> (Say) ³	Tenderfoot leafhopper	1934-36	15
<i>Empoasca</i> sp. ³	Leafhopper	1934-36	290
<i>Ezrinthus obscurinervis</i> (Stal.)	do	1936	1
<i>Macrosiphum</i> sp.	Aphid	1936	20
<i>Macrolestes divinus</i> (Uhl.)	Six-spotted leafhopper (a. n. o.)	1934-36	185
<i>Micralis calva</i> (Say)	Treehopper	1934	1
<i>Norvellina seminuda</i> (Say)	Leafhopper	1934, 1936	4
<i>Philaenus leucophthalmus</i> (L.)	Spittle bug	1935	105

¹ Listed alphabetically according to orders; under each order, alphabetically by genera.² Also tested for *Aplanobacter stewarti* after feeding for several days on infected corn in cages, but data not included in this summary.⁴ a. n. o. = American name, official; i. e., common name adopted by the American Association of Economic Entomologists.

TABLE 6.—Summary of insects¹ collected in the field in 1934-37 and tested for *Aplanobacter stewarti*—ContinuedINSECTS FROM WHICH *A. STEWARTI* WAS NOT ISOLATED—Continued

Order and scientific name	Common name	Years tested	Insects tested
Homoptera—Continued.			
<i>Phlepsius irroratus</i> (Say)	Irrorate leafhopper	1935-36	Number 2
<i>Polyamia inimica</i> (Say)	Inimical leafhopper	1934-36	5
<i>Scaphytopius cinereus</i> (O. and B.)	Leafhopper	1936	1
<i>Scaphytopius frontalis</i> (Van D.)	do	1935-36	2
<i>Sloboera tricarinata</i> (Say)	Fulgorid	1935	5
Lepidoptera:			
<i>Heliothis armigera</i> (Hbn.) (larvae)	Corn earworm (a. n. o.)	1937	20
Orthoptera:			
<i>Conocephalus fasciatus</i> (DeG.)	Grasshopper (nymph)	1935	1
Thysanoptera:			
<i>Aeolothrips fasciatus</i> (L.)	Thrips	1936	5
<i>Aeolothrips fasciatus</i> (L.)		1934	50
<i>Anaphothrips obscurus</i> (Müll.)			

¹ Listed alphabetically according to orders; under each order, alphabetically by genera.CHAETOCNEMA PULICARIA¹¹

ABUNDANCE AND DISTRIBUTION OF INFESTED ADULTS

The corn flea beetle (*Chaetocnema pulicaria*), as previously reported, appears to be the most important insect vector of the wilt organism among the species tested. From the data obtained to date, it also appears to be the only species of importance that harbors the organism over winter and carries it to the young corn in the spring.

Chaetocnema pulicaria usually occurs in much greater abundance on corn throughout most of the season than any of the other insects from which *Aplanobacter stewarti* has been isolated (9). During 1934, 4,790 specimens from 29 localities and 12 host plants were used in isolation tests; in 1935, 5,588 specimens from 45 localities and 10 hosts; in 1936, 3,809 specimens from 20 localities and 5 hosts; and in 1937, 4,426 specimens from 26 localities and 8 hosts; making a total of 18,613 specimens of *C. pulicaria* tested in the 4 years. Table 7 gives in detail the percentages of *C. pulicaria* infested with *A. stewarti* under field conditions at the Arlington farm during the 4-year period 1934-37. *C. pulicaria* was much less abundant in the field in 1936 than in the other 3 years. In the spring and early summer, the percentage of heavily infested beetles was slightly lower than in mid-summer and fall, indicating that as the number of infected corn plants increased, the proportion of beetles carrying an abundance of the organisms also increased. The seasonal life history of this insect is not yet well enough known to correlate the fluctuation in the proportion of adults infested with the appearance of new broods.

The data from collections of *Chaetocnema pulicaria* at the Arlington farm for the period 1934-37 are summarized in table 8.

The data presented in table 8 show that the proportion of infested *Chaetocnema pulicaria* obtained from sweet corn was consistently higher than that obtained from other hosts. The larger percentage of infested *C. pulicaria* found on sweet corn at the Arlington farm in 1936 than in 1934 and 1935 was probably due to the fact that in 1936 collections from this host were made late in the season only, when wilt was more abundant in the field. The percentage of *C. pulicaria* infested with *Aplanobacter stewarti* collected from sweet corn in 1937 was low, probably because 100 of the 225 adults tested were collected

¹¹ Coleoptera, Chrysomelidae.

May 25 from sweet corn transplanted from the greenhouse to a spot located several hundred yards from infected corn of the previous season. Only 2 percent of these 100 beetles yielded *A. stewarti*. If this collection is omitted from the calculations, the remaining 125 adult *C. pulicaria*, all collected on infected sweet corn after July 18, will yield an average of 46.4 percent infested with *A. stewarti*. The percentages, by months, of *C. pulicaria* found to be carrying the wilt organism internally at the Arlington farm during the 4-year period are likewise shown in table 8.

TABLE 7.—Percentage of *Chaetocnema pulicaria* infested with *Aplanobacter stewarti* under field conditions at the Arlington Experiment Farm, Arlington, Va.

Date collected	Host	Adults tested by isolation			Proportion yielding <i>A. stewarti</i>	Date collected	Host	Adults tested by isolation			Proportion yielding <i>A. stewarti</i>
		No.	No.	Pct.				No.	No.	Pct.	
1934						1935					
May 1	Grass	18	2	11.1	July 8	Corn (sweet)	99	49	49.4		
14	Corn (sweet)	85	0	0	15	do	100	59	59.0		
16	Grass	4	1	25.0	22	do	100	49	49.0		
16	Wheat	57	10	17.5	Sept. 25	Alfalfa	100	31	31.0		
16	Corn (sweet)	54	10	18.5	27	Grass	100	32	32.0		
18	do	83	11	13.2	Oct. 28	Sudan grass	110	8	7.2		
June 18	Panic grass	100	0	0	Nov. 5	Alfalfa	110	35	31.8		
20	Corn (field)	50	22	44.0	5	Grass	100	21	21.0		
20	do	50	20	40.0		Total or average	1,765	493	27.9		
July 9	Corn (sweet)	100	75	75.0	1936						
31	do	100	38	38.0	Mar. 25	Grass	104	25	24.0		
Aug. 1	Sudan grass	100	19	19.0	Apr. 15	do	100	13	13.0		
8	do	66	8	12.1	May 13	do	70	8	11.4		
10	Corn (sweet)	90	45	50.0	June 2	Corn (field)	94	20	21.2		
10	Panic grass	10	2	20.0	2	Panic grass	53	0	0		
15	Alfalfa	100	9	9.0	July 2	Corn (field)	54	1	1.8		
19	Corn (sweet)	100	36	36.0	14	do	100	2	2.0		
28	do	100	47	47.0	27	do	48	7	14.5		
Sept. 5	do	100	58	58.0	Aug. 19	Corn (sweet)	100	51	51.0		
11	Sudan grass	100	6	6.0	Sept. 8	do	95	51	53.6		
19	Corn (sweet)	46	26	56.5	23	do	60	43	71.6		
19	Sudan grass	47	1	2.1	Oct. 19	Grass	100	31	31.0		
Oct. 2	do	52	8	15.3	Nov. 2	do	100	38	38.0		
22	Alfalfa and grass	100	3	3.0		Total or average	1,078	290	26.9		
Nov. 5	Grass	105	7	6.6	1937						
22	do	60	9	15.0	Jan. 8	Grass	100	30	30.0		
	Total or average	1,877	473	25.2	Mar. 25	do	23	4	17.3		
1935						Apr. 6	do	100	26	26.0	
Mar. 22	Grass	100	7	7.0	May 25	Corn (sweet)	100	2	2.0		
25	do	100	6	6.0	June 23	Corn (field)	100	2	2.0		
30	do	100	2	2.0	July 19	Corn (sweet)	50	19	38.0		
Apr. 25	do	97	9	9.2	19	Grass	50	6	12.0		
May 14	do	38	6	15.7	Aug. 11	Corn (sweet)	60	29	48.3		
June 28	Corn (sweet)	100	28	28.0	11	Grass	40	4	10.0		
3	do	63	32	50.7	Sept. 15	Corn (sweet)	15	10	66.6		
3	do	31	16	51.6	15	Grass	87	23	26.4		
3	do	5	3	60.0	Oct. 12	do	50	12	24.0		
10	do	100	45	45.0	12	Alfalfa (grassy)	50	6	12.0		
15	do	84	1	1.2	Nov. 8	Weeds, grass, etc.	85	19	22.3		
15	do	31	0	0		Total or average	910	192	21.0		
26	do	94	54	57.4							
27	do	1	0	0							

♀ Female.

♂ Male.

Although these data are incomplete they indicate that higher percentages of these beetles were carrying the wilt organism during July, August, and September than during other months of the year. It seems clear that while the percentages of infested *Chaetocnema pulicaria* at the Arlington farm early in the season were higher in 1936 than in 1935, midseason percentages were lower, and late-season percentages again were higher.

TABLE 8.—*Proportion of adults of Chaetocnema pulicaria, infested with Aplanobacter stewarti, collected from sweet corn and other hosts at the Arlington Experiment Farm, Arlington, Va., 1934–37*

Host and year	Period of collection	Total adults	Adults infested										
			Aver- age	Jan.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.
			No.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
Sweet corn:													
1934	May 14 to Sept. 19	908	40.3				9.4	40.0	56.5	44.1	57.5		
1935	May 28 to July 22	808	41.5				28.0	36.9	52.5				
1936	Aug. 19 to Sept. 23	255	56.8							51.0	60.6		
1937	May 25 to Sept. 15	225	26.6				2.0		38.0	48.3	66.6		
Other hosts:													
1934	May 1 to Sept. 19	669	12.1				16.4	14.6	5.8	13.8	4.7	7.2	9.6
1935	Mar. 22 to Nov. 5	955	16.4		5.0	9.2	15.7				51.5	7.2	26.6
1936	Mar. 25 to Nov. 2	823	17.6				24.0	13.0	11.4	21.2	3.9	31.0	38.0
1937	Jan. 8 to Nov. 8	685	19.2	30.0	17.3	26.0		2.0	12.0	10.0	26.4	18.0	22.3

¹ See text p. 664 for explanation of low figure.

Table 9 gives detailed information on the percentage of *Chaetocnema pulicaria* infested with *Aplanobacter stewarti* under field conditions when collected from localities other than the Arlington farm during the 4-year period 1934–37. Excluding collections of 10 adults of *C. pulicaria* or less, high percentages (30 percent or more) of infested beetles collected from localities other than the Arlington farm for the 4-year period are shown in table 10.

TABLE 9.—*Percentage of Chaetocnema pulicaria, from localities other than the Arlington Experiment Farm, Arlington, Va., infested with Aplanobacter stewarti*

1934

Date collected	Host	State	Adults tested	Proportion yielding <i>A. stewarti</i>	
				Number	Percent
May 5	Oats	South Carolina	112	0	0.0
May 7	Corn	do	145	0	.0
May 8	Weeds	North Carolina	19	0	.0
May 11	Oats	Virginia	59	0	.0
June 6	do	Maryland	67	11	16.4
Do	Corn	do	50	3	6.0
Do	do	New Jersey	8	0	.0
Do	Tomato	do	72	0	.0
June 10	Oats	do	3	0	.0
June 22	Corn	Virginia	50	14	28.0
Do	do	do	50	8	16.0
June 27	do	Pennsylvania	16	4	25.0
Do	do	do	5	3	60.0
July 2	do	Virginia	50	8	16.0
July 11	do	do	54	30	55.5
Do	do	do	50	3	6.0
July 17	do	New Jersey	85	18	21.1
Do	do	Delaware	97	19	19.5
July 18	do	Connecticut	4	0	.0
July 20	do	New York	5	0	.0
July 21	do	Pennsylvania	68	28	41.1
Do	do	do	100	15	15.0
Do	do	do	100	2	2.0
Aug. 21	do	Virginia	100	40	40.0
Do	do	North Carolina	27	5	18.6
Aug. 30	do	Virginia	100	29	29.0
Sept. 6	do	do	100	16	16.0
Sept. 10	do	Ohio	2	0	.0
Oct. 8	Corn and grass	Missouri	110	18	16.3
Oct. 10	Corn	Kansas	160	7	4.3
Oct. 15	Rye and alfalfa	Illinois	97	38	39.1
Total or average			1,965	319	16.2

TABLE 9.—*Percentage of Chaetocnema pulicaria, from localities other than the Arlington Experiment Farm, Arlington, Va., infested with Aplanobacter stewarti*
Continued

1935

Date collected	Host	State	Adults tested	Proportion yielding <i>A. stewarti</i>	
				Number	Percent
April 2	Grass	Virginia	110	11	10.0
Do	do	do	3♂	0	.0
Do	do	do	1♀	0	.0
Do	do	do	98	5	5.1
Do	do	do	1♂	0	.0
Do	do	do	2♀	0	.0
April 19	Bluegrass	Missouri	6	1	16.6
April 20	do	Ohio	83	2	2.4
Do	Grass	Indiana	111	7	6.3
Do	do	Ohio	8	0	.0
April 28	do	Virginia	117	7	5.9
May 7	do	North Carolina	100	2	2.0
May 8	Oats	Virginia	30	1	3.3
Do	do	North Carolina	55	2	3.6
Do	Grass	do	16	2	12.5
Do	do	South Carolina	2	0	.0
Do	Alfalfa	do	1	0	.0
Do	Corn	do	49	0	.0
May 9	do	do	52	1	1.9
Do	do	do	84	9	10.7
May 10	do	Virginia	67	12	17.9
May 11	Grass	do	11	1	5.2
Do	Alfalfa	do	7	0	.0
May 15	Oats	Maryland	100	22	22.0
Do	Grass	do	3	2	66.6
Do	Wheat	Delaware	25	3	12.0
May 16	Corn	New Jersey	100	36	36.0
Do	Oats	do	99	13	13.1
May 17	Grass	Connecticut	2	0	.0
May 21	Corn	Maryland	55	0	.0
May 22	Grass	Pennsylvania	100	31	31.0
Do	Oats	do	9	3	33.3
May 31	Corn	Virginia	100	13	13.0
June 6	do	do	6	2	33.3
June 7	do	North Carolina	7	1	14.2
Do	do	do	14	1	7.1
June 11	do	Virginia	140	28	20.0
June 18	Oats	Pennsylvania	120	24	20.0
June 24	Corn	do	23	1	4.3
Do	do	New Jersey	1	0	.0
July 1	do	Virginia	100	34	34.0
July 12	do	do	100	28	28.0
July 16	do	New Jersey	100	43	43.0
July 17	do	New York	100	52	52.0
Do	do	do	28	12	42.8
July 18	do	Connecticut	47	6	12.7
July 19	do	New York	13	1	7.6
July 20	do	Pennsylvania	60	8	13.3
Do	do	do	60	6	10.0
Do	do	do	47	14	29.7
Aug. 14	do	do	24	6	25.0
Aug. 16	do	New York	28	3	10.7
Aug. 17	do	Connecticut	45	10	22.2
Aug. 18	do	New York	55	31	56.3
Sept. 24	Alfalfa	Virginia	100	37	37.0
Oct. 8	Corn	do	98	29	29.5
Oct. 12	Alfalfa	West Virginia	100	27	27.0
Oct. 13	do	Illinois	64	6	9.3
Do	Clover	do	40	13	32.5
Oct. 14	Grass	Missouri	100	5	5.0
Oct. 15	Alfalfa	Kansas	100	3	3.0
Oct. 19	Grass	Indiana	100	10	10.0
Do	do	Ohio	110	20	18.1
Nov. 5	do	Virginia	100	29	29.0
Total or average			3,645	686	18.8

1936

Feb. 26	Rye	Virginia	80	4	5.0
Mar. 24	do	do	110	3	2.7
Apr. 13	do	do	112	7	6.2
Apr. 20	Bluegrass	Ohio	68	4	5.8
Apr. 28	Grass	Virginia	39	3	7.6
Apr. 29	Rye	do	93	4	4.3

TABLE 9.—Percentage of *Chaetocnema pulicaria*, from localities other than the Arlington Experiment Farm, Arlington, Va., infested with *Aplanobacter stewarti*—Continued

1936—Continued

Date collected	Host	State	Adults tested	Proportion yielding <i>A. stewarti</i>	
				Number	Percent
Apr. 29	Grass	North Carolina	100	6	6.0
May 6	Corn	South Carolina	93	3	3.2
Do	Rye	Virginia	88	22	25.0
May 15	Corn	Indiana	3	0	.0
Do	Grass	do	5	0	.0
May 25	Corn	Pennsylvania	21	1	4.7
Do	do	do	2	2	100.0
Do	do	Ohio	4	1	25.0
May 26	do	Virginia	100	10	10.0
Do	do	Indiana	11	0	.0
May 27	do	New York	46	3	6.5
June 4	do	Ohio	1	0	.0
June 9	do	Virginia	100	41	41.0
June 15	do	Ohio	1	0	.0
June 23	do	Indiana	74	0	.0
June 25	do	Ohio	1	0	.0
July 6	do	do	1	0	.0
July 16	do	do	6	0	.0
July 21	?	New York	19	3	15.7
Do	Corn	Indiana	61	1	1.63
July 22	do	Virginia	86	40	46.5
July 23	do	do	50	23	46.0
Do	do	do	50	20	40.0
July 28	do	Ohio	30	0	.0
July 29	Corn and yellow bristle grass.	Pennsylvania	48	0	.0
July 31	Corn	do	50	0	.0
Aug. 7	do	Ohio	70	0	.0
Aug. 12	do	New York	40	19	47.5
Do	do	Virginia	50	16	32.0
Aug. 17	do	Ohio	113	0	.0
Aug. 25	do	Pennsylvania	92	4	4.3
Aug. 26	do	New York	5	0	.0
Aug. 27	do	do	100	49	49.0
Do	do	Ohio	100	0	.0
Aug. 28	do	Delaware	100	39	39.0
Sept. 1	do	Indiana	4	0	.0
Do	do	do	100	60	60.0
Sept. 8	do	Ohio	100	0	.0
Oct. 6	Rye	New York	75	33	44.0
Oct. 20	Grass	Indiana	53	14	26.4
Nov. 2	?	Virginia	100	18	18.0
Nov. 7	Bluegrass	Ohio	76	0	.0
Dec. 28	do	Indiana	8	1	12.5
Dec. 30	Grass	do	3	1	33.3
Total or average			2,742	455	16.5

1937

Jan. 10		Virginia	100	13	13.0
Apr. 21	Grasscs	do	50	9	18.0
Do	do	do	50	11	22.0
May 3	Grass	Ohio	100	7	7.0
May 7	do	Indiana	38	2	5.2
May 8	do	do	21	2	9.5
May 12	do	Ohio	4	0	.0
May 13	Field corn	North Carolina	100	10	10.0
May 29	Sweet corn	New Jersey	63	32	50.7
Do	Field corn	Delaware	100	8	8.0
Do	Sweet corn	New Jersey	100	27	27.0
Do	Field corn	Maryland	88	0	.0
June 1	Sweet corn	New York	54	22	40.7
June 3	do	Pennsylvania	30	1	3.3
June 4	Field corn	do	45	0	.0
Do	do	do	50	2	4.0
June 9	Sweet corn	New Jersey	100	71	71.0
June 15	Field and sweet corn	Ohio	100	1	1.0
June 17	Sweet corn	New Jersey	100	64	64.0
June 21	Dent corn	Indiana	69	30	43.4
June 24	Sweet corn	New Jersey	100	63	63.0
June 26	Field corn	Pennsylvania	100	4	4.0
June 29	Sweet corn	New York	48	26	54.1
July 8	Field corn	New Jersey	100	48	48.0

TABLE 9.—Percentage of *Chaetocnema pulicaria*, from localities other than the Arlington Experiment Farm, Arlington, Va., infested with *Aplanobacter stewarti*—Continued

1937—Continued

Date collected	Host	State	Adults tested	Proportion yielding <i>A. stewarti</i>	
			Number	Number	Percent
July 19	Sweet corn	Indiana	25	1	4.0
Do	Dent corn	do	32	5	15.6
July 21	do	do	84	25	29.7
July 22	Field corn	New Jersey	86	64	74.3
July 23	Sweet and field corn	Ohio	93	1	1.0
July 26	Sweet corn	New York	100	75	75.0
Aug. 10	do	Indiana	26	12	46.1
Do	Dent corn	do	97	10	10.3
Aug. 11	Sweet corn	do	10	2	20.0
Aug. 13	do	Ohio	100	49	49.0
Aug. 17	Field corn	Pennsylvania	100	18	18.0
Do	do	do	48	6	12.5
Do	Grassy corn	do	21	7	33.3
Aug. 18	Sweet corn	New York	57	10	17.5
Do	do	do	17	6	35.2
Aug. 19	Corn	do	10	3	33.3
Do	Sweet corn	do	9	4	44.4
Do	do	do	45	21	46.6
Aug. 20	Field corn	New Jersey	100	54	54.0
Aug. 28	Sweet corn	New York	6	1	16.6
Sept. 2	do	do	93	70	75.2
Sept. 18	Bluegrass	Ohio	100	37	37.0
Sept. 20	Sweet corn	do	100	32	32.0
Sept. 23	Grass	New Jersey	88	43	48.8
Oct. 1	Sweet corn	Indiana	21	11	52.3
Do	Dent corn	do	77	15	19.4
Oct. 2	Sweet corn	do	50	14	28.0
Oct. 5	Rye	New York	100	50	50.0
Oct. 11	Sweet corn	Ohio	100	21	21.0
Total or average			3,505	1,120	31.9

TABLE 10.—Percentage (30 percent or more) of infested *Chaetocnema pulicaria* collected in localities other than the Arlington Experiment Farm, Arlington, Va., 1934-37

Date	Locality	Infested beetles	Date	Locality	Infested beetles
1934		Percent	1936		Percent
July 11	Charlottesville, Va.	55.0	Aug. 27	Hempstead, N. Y.	49.0
Aug. 21	Franklin, Va.	40.0	Oct. 6	do	44.4
July 21	Hamburg, Pa.	41.1	Sept. 1	La Fayette, Ind.	60.0
Oct. 15	Madison County, Ill.	39.1	1937		
1935			May 29	Burlington, N. J.	50.7
July 1	Falls Church, Va.	34.0	June 9	do	71.0
July 12	Norfolk, Va.	38.0	June 17	do	64.0
Sept. 24	Falls Church, Va.	37.0	June 24	do	63.0
May 22	Gettysburg, Pa.	31.0	July 8	do	48.0
May 16	Burlington, N. J.	36.0	July 22	do	74.4
July 16	do	43.0	Aug. 20	do	54.0
July 17	Hicksdale, N. Y.	52.0	Sept. 23	do	48.8
Aug. 18	Huntington Station, N. Y.	42.8	June 1	Hempstead, N. Y.	40.7
Aug. 17	Yonkers, N. Y.	56.3	June 29	do	54.1
Oct. 13	Hamel, Ill.	32.5	July 26	do	75.0
1936			Aug. 18	Watkins Glen, N. Y.	35.2
June 9	Falls Church, Va.	41.0	Aug. 19	Schenectady, N. Y.	46.6
July 22	Holland, Va.	46.5	Sept. 2	Hempstead, N. Y.	75.2
July 23	Norfolk, Va.	46.0	Oct. 5	do	50.0
Aug. 12	Surry, Va.	40.0	Aug. 17	Williamsport, Pa.	33.3
Aug. 12	Holland, Va.	32.0	Aug. 13	Toledo, Ohio	49.0
Aug. 28	Glasgow, Del.	39.0	Sept. 18	Maricetta, Ohio	37.0
Aug. 12	Mineola, N. Y.	47.5	Sept. 20	Toledo, Ohio	32.0
			June 21	La Fayette, Ind.	43.4
			Aug. 10	do	46.1
			Oct. 1	do	52.3

It would appear from these data that higher percentages of *Chaetocnema pulicaria* were carrying *Aplanobacter stewarti* in 1937, es-

pecially during August and September, than during the same months of the 3 preceding years. However, the collections during the 4-year period upon which these data are based were made at different times and from different localities and are therefore not entirely comparable.

Heavy seedings and practically pure cultures of the wilt organism were obtained in 83 percent of the 1,198 isolations of *Aplanobacter stewarti* from *Chaetocnema pulicaria* in 1935, in 76 percent of the 743 isolations made in 1936, and in 87 percent of the 1,315 isolations made in 1937. Less than 20 percent of the plates had from 1 to several colonies of *A. stewarti* in a mixture of other organisms.

OVERWINTERING OF *Aplanobacter stewarti*

As previously reported (4, 9) about 19 percent of 175 adults of *Chaetocnema pulicaria* collected at the Arlington farm before corn came up in the spring of 1934 were found to be heavily infested with *Aplanobacter stewarti* when tested individually for this organism. Sweet corn plants in cages in the greenhouse developed typical symptoms of the wilt disease after adults of *C. pulicaria* from similar lots had fed on the leaves. Additional feeding tests of a similar nature were made during either March or April of the 3 years following, and similar results were obtained in each instance. Isolations of *A. stewarti* during 1935-37 from overwintered adults of *C. pulicaria* early in the season before corn had come up in the field and from adult beetles going into hibernation in October and November continued to furnish evidence of the overwintering of *A. stewarti* in this insect. The results of these early- and late-season isolations are given in table 11. The figures shown are not entirely comparable since the collections upon which they are based were not made at the same time and from the same localities and hosts from year to year. They indicate, however, that larger percentages of *C. pulicaria* about to hibernate were carrying the wilt organism in the fall of 1936 and 1937 than during the late fall of the two preceding years. Table 11 also indicates that high percentages of infested beetles coming out of hibernation in the spring apparently follow high percentages going into hibernation in the fall. In years when few infested beetles go into hibernation in the fall, ring tests have shown similar low percentages.

TABLE 11.--Proportion of adults of *Chaetocnema pulicaria* harboring *Aplanobacter stewarti* in the spring and fall 1934-37

Year	Spring			Fall		
	Localities	Adults tested	Proportion yielding wilt organism	Localities	Adults tested	Proportion yielding wilt organism
	Number	Number	Percent	Number	Number	Percent
1934.....	1	175	19.3	4	684	13.1
1935.....	16	1,427	9.0	10	1,132	18.2
1936.....	6	964	10.2	5	504	26.5
1937.....	8	586	17.7	5	533	27.7

ARLINGTON EXPERIMENT FARM

Year	Localities	Adults tested	Proportion yielding wilt organism	Localities	Adults tested	Proportion yielding wilt organism
1934.....	1	175	19.3	1	317	8.5
1935.....	1	397	6.0	1	320	20.0
1936.....	1	204	18.6	1	200	34.5
1937.....	1	223	26.9	1	185	20.0

An attempt was made, insofar as possible, to make the collections from which these data were obtained in the same areas each season. However, this was not always possible and frequently there was much variation in the percentage of *Chaetocnema pulicaria* yielding the wilt organism from areas near infected corn of the previous season and from areas at distances of one-half mile or more from the corn. The foregoing data appear to indicate a considerable increase in the percentage of beetles carrying the wilt organism in the fall of 1935 and in the spring of 1936 and a still greater increase in the fall of 1936 and in the spring of 1937. The late development of wilt at the Arlington farm during 1936 probably was responsible for the higher percentages of infested beetles in the fall than in midsummer in 1936, and these higher percentages going into hibernation were in turn probably responsible for the higher percentages coming out of hibernation the following spring.

Aplanobacter stewarti was isolated from specimens of *Chaetocnema pulicaria* collected at the Arlington farm every month of the year except February during the 4-year period. Collections of *C. pulicaria* were made during the winter, whenever weather conditions were favorable for the beetles to become active. One lot of 80 individuals of this species, collected February 26, 1936, at Norfolk, Va., was used for isolation of *A. stewarti*, and 5 percent of these adults yielded the organism. As table 9 shows, *A. stewarti* was isolated from overwintering adults of *C. pulicaria* from every section of the country in which wilt had been at all prevalent.

PROPORTION OF EACH SEX CARRYING THE WILT ORGANISM AND MAXIMUM INFESTATION OF ADULTS

In 1935 the sex of the adults was determined in 2 experiments in which field-collected specimens of *Chaetocnema pulicaria* were tested by isolation in the usual manner. In 1 test 16 isolations of *Aplanobacter stewarti* were obtained from 31 males and 32 isolations from 63 females, 51.6 percent as compared with 50.7 percent. These adults were collected at the Arlington farm, June 3, from sweet corn transplanted from the greenhouse in April and May. This test indicated no significant difference between the sexes in the proportion of adults carrying the wilt organism. A second test with adults collected from the same plants on June 15 yielded 1 culture of *A. stewarti* from 84 individual females and none from 31 males. An additional test was made July 3, 1935, in which a total of 103 individuals of *C. pulicaria* that had been confined to infected corn in cages for 5 days were tested in the usual manner for *A. stewarti*, and 86, or 83.4 percent, yielded the organism. The sex of 87 of these 103 beetles was determined as they were crushed in the broth, and 36 out of 46 females, or 78.2 percent, and 39 out of 41 males, or 95.1 percent, yielded the wilt organism. This was the highest percentage of *C. pulicaria* ever recorded as carrying *A. stewarti* internally. During 1934 (9) only 30.7 to 68 percent, or a weighted average of 55.6 percent, of the adults of *C. pulicaria* yielded *A. stewarti* in similar tests. The reason for the difference in the number of isolations of *A. stewarti* obtained in the first 2 tests is not clear, but the difference may have been due to the appearance of a new brood of *C. pulicaria* that had not become infested with the organism prior to being used for isolation.

The maximum infestation from field collections of adults of *Chaetocnema pulicaria* in 1934 was 75 percent on July 9 (table 7); in 1935, 59 percent on July 15 (excluding the collection of five adults made June 3); in 1936, 71.6 percent on September 23 (excluding the two adults collected May 25); and in 1937, 75.2 percent on September 2 (table 9). The highest percentages of beetles carrying the wilt organism were from adults collected on or near infected corn.

CHAETOCNEMA DENTICULATA¹²

Among the numerous species of insects tested for the bacterial wilt organism during the 4-year period 1934-37, *Chaetocnema denticulata* appears to be second in importance as a vector of *Aplanobacter stewarti* under field conditions. In 1934, 366 isolations from 905 specimens of *C. denticulata* yielded *A. stewarti* in 26 instances (table 6); in 1935, 112 isolations from 597 specimens yielded 4 cultures of *A. stewarti*; in 1936, 82 isolations from 95 specimens yielded only 2 cultures; and in 1937, 289 isolations from 409 specimens yielded 12 cultures. Table 12 summarizes the results of isolations from adults of *C. denticulata* collected at the Arlington farm to determine the wilt-carrying possibilities of this species, and table 13 gives similar data for collections from localities other than the Arlington farm.

At the Arlington farm during 1934-37, a total of 439 isolations were made from 927 adults of *Chaetocnema denticulata*, and *Aplanobacter stewarti* was obtained from 23 of these isolations. Of particular interest in connection with these studies was the fact that 17 of the 23 cultures were secured during August and September of 1934, when wilt apparently was most prevalent in the field. No cultures of *A. stewarti* were obtained in 1936, although 56 isolations from 59 adults were made. One culture was obtained from 25 isolations from a single adult *C. denticulata* collected January 8, 1937.

From localities other than the Arlington farm, during the 4-year period, 410 isolations were made from 1,079 adults of *Chaetocnema denticulata*, and 21 cultures of *Aplanobacter stewarti* were obtained as follows: 9 out of 192 in 1934, from adults collected from corn in Virginia during August and September; 1 out of 75 in 1935, from an adult collected from corn in Virginia; 2 out of 26 in 1936, one from corn and the other from rye, at Mineola, Long Island, N. Y.; and 9 out of 117 in 1937, from adults collected from sweet corn and grass (4 from sweet corn and grass in New Jersey, 4 from sweet corn at Mineola, and 1 from grass at Marietta, Ohio). While *C. denticulata* does carry the wilt organism, it is apparently not a very close second in importance to *C. pulicaria* in the dissemination of wilt in the field. Moreover, it is very doubtful whether the organism ever overwinters in adults of *C. denticulata*. No isolations of the wilt organism have been obtained in March and April, although 3 isolations were made from *C. denticulata* collected from rye and grass in the fall and winter: One, November 5, 1935; another, October 6, 1936; and another, January 8, 1937. Several early and midseason isolations of what appeared to be *A. stewarti* on plates poured from broth suspensions of adult *C. denticulata* were not infectious.

¹² Coleoptera, Chrysomelidae.

TABLE 12.—*Number of Chaetocnema denticulata infested with Aplanobacter stewartii at the Arlington Experiment Farm, Arlington, Va.*

1934

Date collected	Host	Insects per isolation	Isolations	Type of culture	Proportion of isolations yielding <i>A. stewartii</i>	
		Number	Number		Number	Percent
Apr. 4	Alfalfa	2	1	C	0	0.0
Apr. 30	Rye	6	1	B	0	.0
May 16	Corn	1	1	C	0	.0
May 18	Alfalfa	8	1	C	0	.0
June 25	Corn	1	5	C	0	.0
July 31	do	1	2	2A	0	.0
Aug. 3	do	107	1	B	0	.0
Aug. 10	do	9	1	C	0	.0
Do	Grass	44	1	A	0	.0
Do	do	42	1	A	1	100.0
Aug. 15	Alfalfa	15	1	B	0	.0
Do	do	54	1	B	1	100.0
Aug. 17	Grass	1	115	{ 7A 8B	5 1	5.2
Do	do	18	1	C	0	.0
Aug. 19	Corn	1	10	1B	0	.0
Do	do	5	4	{ 3C 1B	0 1	.0 25.0
Do	do	10	2	2B	2	100.0
Aug. 28	Grass	1	4	1B	1	25.0
Sept. 5	Corn	1	1	B	1	100.0
Do	do	1	1	A	1	100.0
Sept. 11	Sudan grass	1	9	{ 2A 1B	1 0	11.1
Do	do	2	1	C	0	.0
Do	do	3	1	C	0	.0
Do	do	5	1	C	0	.0
Do	do	5	2	2A	2	100.0
Sept. 19	Corn	5	1	A	0	.0
Do	Sudan grass	7	1	A	0	.0
Do	do	6	1	C	0	.0
Oct. 2	do	14	1	C	0	.0
		15	1	B	0	.0
Total or average		2 560	174		17	9.7

1935

Mar. 21	Rye	1	4	C	0	0.0
Mar. 23	Grass	1	2	C	0	.0
Apr. 24	do	1	1	C	0	.0
May 14	do	6	1	B	0	.0
June 3	Corn	1	1	C	0	.0
June 26	do	1	3	2A	2	66.6
June 27	do	1	4	C	0	.0
June 28	do	1	5	C	0	.0
July 5	do	12	1	C	0	.0
Do	do	14	1	C	0	.0
July 6	do	66	1	C	0	.0
July 8	do	2	1	C	0	.0
July 23	do	1	1	A	0	.0
Do	do	1	1	C	0	.0
Sept. 26	Grass	1	9	1A	0	.0
Nov. 5	do	1	1	A	1	100.0
Total or average		132	37		3	8.1

1936

Mar. 25	Grass	1	6	C	0	0.0
Apr. 15	do	1	42	1B	0	.0
July 2	Panic grass	1	5	C	0	.0
July 27	do	1	2	C	0	.0
Nov. 3	Grass	4	1	C	0	.0
Total or average		59	56		0	.0

See footnotes at end of table.

TABLE 12.—*Number of Chaetocnema denticulata infested with Aplanobacter stewarti at the Arlington Experiment Farm, Arlington, Va.—Continued*

1937

Date collected	Host	Insects per isolation	Isolations	Type of culture	Proportion of isolations yielding <i>A. stewarti</i>	
		Number	Number		Number	Percent
Jan. 8.....	Grass.....	1	25	A	1	4.0
July 19.....	do.....	1	50	C		
Aug. 11.....	Sweet corn.....	1	25	A	1	4.0
Do.....	Grasses.....	1	25	C		
Sept. 15.....	Sweet corn.....	1	1	A	1	100.0
Do.....	Grass.....	1	1	C		
Oct. 12.....	do.....	1	2	C		
Do.....	Grassy alfalfa.....	1	7	C		
Do.....	do.....	5	1	C		
Nov. 8.....	Weeds, etc.....	1	35	C		
Total or average.....		176	172		3	1.7
Grand total.....		927	439		23	

¹ A = Typical colonies of *Aplanobacter stewarti*; B = atypical yellow colonies; C = no colonies.² Total number of insects = insects per isolation × isolations.TABLE 13.—*Number of Chaetocnema denticulata, from localities other than Arlington, Va., infested with Aplanobacter stewarti*

1934

Date collected	Host	State	Insects per isolation	Isolations	Type of culture	Proportion of isolations yielding <i>A. stewarti</i>	
			Number	Number		Number	Percent
May 5.....	Oats.....	Georgia.....	18	1	A	0	0.0
May 11.....	Woods.....	Virginia.....	1	1	B	0	.0
June 6.....	Corn.....	New Jersey.....	1	1	C	0	.0
Do.....	Oats.....	Maryland.....	1	4	2B	0	.0
July 11.....	Corn.....	Virginia.....	1	41	2B	0	.0
Do.....	do.....	do.....	1	50	{ 4A 7B	0	.0
July 18.....	do.....	Connecticut.....	10	1	B	0	.0
July 19.....	do.....	New York.....	19	1	B	0	.0
July 20.....	do.....	do.....	1	41	11B	0	.0
July 21.....	do.....	Pennsylvania.....	2	1	C	0	.0
Aug. 2.....	do.....	North Carolina.....	106	1	C	0	.0
Do.....	do.....	Virginia.....	1	16	{ 3A 3B	1	18.7
Do.....	do.....	do.....	2	1	A	0	.0
Aug. 30.....	do.....	do.....	1	14	6A	5	35.7
Do.....	do.....	do.....	3	1	C	0	.0
Sept. 6.....	do.....	do.....	1	7	2A	1	14.2
Oct. 15.....	Rye.....	Illinois.....	1	10	1B	0	.0
Total or average.....			2 345	192		9	4.7

1935

Apr. 2.....	Grass.....	Virginia.....	1	1	A	0	0.0
Apr. 19.....	do.....	Missouri.....	1	27	C	0	.0
Apr. 20.....	do.....	Indiana.....	1	1	C	0	.0
May 7.....	do.....	North Carolina.....	1	1	C	0	.0
May 8.....	Oats.....	do.....	3	1	C	0	.0
Do.....	Grass.....	do.....	50	1	A	0	.0
Do.....	Corn.....	South Carolina.....	28	1	B	0	.0
Do.....	do.....	do.....	3	1	C	0	.0
Do.....	do.....	do.....	2	1	C	0	.0
May 9.....	Potato.....	do.....	2	1	C	0	.0
May 10.....	Corn.....	North Carolina.....	50	1	C	0	.0
May 11.....	Grass.....	Virginia.....	4	1	C	0	.0
May 15.....	Oats.....	Maryland.....	2	1	C	0	.0
May 16.....	Corn.....	New Jersey.....	10	1	A	0	.0
May 18.....	Grass.....	Connecticut.....	1	1	C	0	.0
May 19.....	Corn.....	do.....	2	1	C	0	.0
May 31.....	do.....	Virginia.....	1	1	B	1	100.0
June 6.....	do.....	do.....	1	1	C	0	.0
June 7.....	do.....	North Carolina.....	10	5	C	0	.0
Do.....	do.....	do.....	7	1	C	0	.0
Do.....	do.....	do.....	10	3	B	0	.0
Do.....	do.....	do.....	6	1	B	0	.0

See footnotes at end of table.

TABLE 13.—*Number of Chaetocnema denticulata, from localities other than Arlington, Va., infested with Aplanobacter stewarti*—Continued

1935—Continued

Date collected	Host	State	Insects per isolation	Isolations	Type of culture ¹	Proportion of isolations yielding <i>A. stewarti</i>	
			Number	Number		Number	Percent
June 11	Corn	Virginia	1	1	C	0	.0
June 23	do	Connecticut	1	1	C	0	.0
July 16	do	New Jersey	3	1	C	0	.0
July 17	do	New York	1	1	C	0	.0
July 18	do	Connecticut	10	1	C	0	.0
July 19	do	New York	9	1	C	0	.0
July 20	do	Pennsylvania	1	1	C	0	.0
Aug. 16	do	Massachusetts	53	1	C	0	.0
Aug. 17	do	Connecticut	31	1	C	0	.0
Do	do	do	51	1	C	0	.0
Oct. 8	Grass	Virginia	1	7	C	0	.0
Oct. 13	Alfalfa	Illinois	1	1	C	0	.0
Oct. 14	Grass	Missouri	2	1	C	0	.0
Oct. 15	Alfalfa	Kansas	10	1	C	0	.0
Oct. 19	Bluegrass	Indiana	1	1	C	0	.0
Total or average			2 465	75		1	1.3

1936

Apr. 20	Bluegrass	Ohio	1	4	{ 1B 3C }	0	0.0
Apr. 23	Rye	Virginia	1	6	C	0	.0
Apr. 30	?	North Carolina	1	1	C	0	.0
May 7	Various	Indiana	1	1	C	0	.0
May 13	Corn	do	1	1	C	0	.0
May 26	do	Virginia	1	2	C	0	.0
Do	Red clover	New York	2	1	C	0	.0
Do	Alfalfa	do	1	1	C	0	.0
May 27	Corn	do	1	1	A	1	100.0
July 23	do	Virginia	1	1	C	0	.0
Do	do	do	1	1	C	0	.0
July 29	do	Pennsylvania	1	1	C	0	.0
Aug. 26	do	New York	5	2	2B	0	.0
Sept. 8	do	Ohio	1	1	C	0	.0
Oct. 6	Rye	New York	1	1	A	1	100.0
Nov. 2	?	Virginia	2	1	C	0	.0
Total or average			2 36	26		2	7.6

1937

Jan. 10		Virginia	1	6	C	0	0.0
Jan. 28	Grass	Indiana	1	1	C	0	.0
Apr. 21	do	Virginia	1	3	C	0	.0
May 13	Corn	North Carolina	1	1	C	0	.0
May 29	Sweet corn	New Jersey	1	6	C	0	.0
Do	Field corn	Maryland	1	1	C	0	.0
June 9	Sweet corn	New Jersey	1	4	{ 1A 1B }	1	25.0
July 8	Field corn	do	30	1	C	0	.0
July 19	Sweet corn	Indiana	1	1	C	0	.0
July 21	do	do	1	1	C	0	.0
July 22	Grass	New Jersey	1	32	{ 2A 4B }	2	6.2
Aug. 17	Grassy corn	Pennsylvania	10	4	C	0	.0
Aug. 18	Sweet corn	New York	4	1	C	0	.0
Aug. 19	do	do	6	1	C	0	.0
Aug. 20	Corn	do	10	4	C	0	.0
Do	do	do	8	1	C	0	.0
Do	Field corn	New Jersey	1	1	C	0	.0
Sept. 2	Sweet corn	New York	1	11	6A	4	36.3
Sept. 18	Grass	Ohio	1	10	6B	1	10.0
Sept. 23	do	New Jersey	1	26	4B	1	3.8
Oct. 5	Rye	New York	1	1	C	0	.0
Total or average			2 233	117		9	7.6
Grand total			1,079	410		21	

¹ A=Typical colonies of *Aplanobacter stewarti*; B=atypical yellow colonies; C=no colonies.² Total number of insects=insects per isolation×isolations.

Fifteen of the forty-four isolations of *Aplanobacter stewarti* from field-collected specimens of *Chaetocnema denticulata*, or 34.1 percent, were heavy seedings, as compared with about 80 percent in the case of *C. pulicaria*. The other plates contained only a few colonies of *A. stewarti* or doubtful yellow colonies. Three heavy seedings of what appeared to be *A. stewarti* produced no infection on corn, even after inoculations were repeated. Almost half of the *C. denticulata* specimens from which *A. stewarti* was isolated were collected from hosts other than corn. *C. denticulata* is not so abundant as *C. pulicaria* on corn at the Arlington farm and prefers fall panicum¹³ when it is available, but it ranks next to *C. pulicaria* in number of field-collected adults from which *A. stewarti* was isolated. Apparently, *C. denticulata* does not furnish so favorable an environment for the wilt organism as does *C. pulicaria*. Possibly the organisms become less virulent when associated with *C. denticulata*.

The maximum number of *Chaetocnema denticulata* adults yielding *Aplanobacter stewarti* was obtained in 1935 in a test of 97 adults that had been confined to infected corn for 4 days before the test. Fifty-nine, or 60.8 percent, of 97 adults yielded colonies of *A. stewarti*, as compared with 47.8 percent of 23 *C. denticulata* adults under similar conditions in 1934 (9).

OTHER INSECTS APPARENTLY CARRYING APLANOBACTER STEWARTI

In addition to isolations from *Chaetocnema pulicaria* and from *C. denticulata*, isolations of *Aplanobacter stewarti* were made from each of 12 other species of insects collected in the field during 1934-37, in one or more instances. Whether more isolations of *A. stewarti* would have been obtained from any of the 12 species if larger numbers of each had been tested is problematical. On the basis of the results so far obtained, none of the species from which *A. stewarti* has been isolated, except *C. pulicaria* and *C. denticulata*, can be considered as vectors of any importance under field conditions. The records on the isolations of *A. stewarti* obtained from several of the species discussed in this section would not be presented had not some of these species, after being confined to infected corn in cages for several days, also yielded the wilt organism after being externally disinfected and tested in the usual way. The species involved are treated in the same order in which they are listed in table 6. It may be noted that *A. stewarti* was isolated from specimens of the smut- and pollen-feeding beetles *Phalacrus* sp., *Stilbus apicalis*, and *S. viduus*. Although *A. stewarti* has not been isolated from the pollen cells of corn, the bacteria have been found mixed with pollen and have been isolated from the anther filaments and vascular tissue of the corn tassel (7). Just how *A. stewarti* came to be present in these smut- and pollen-feeding beetles is not apparent, especially since very little wilt occurred in the location where the infected *Phalacrus* specimens were collected and most of the infested specimens of *S. apicalis* and *S. viduus* were collected in late fall long after corn pollen was available.

The types of cultures obtained from the 12 species, other than *Chaetocnema pulicaria* and *C. denticulata*, indicate that these species may occasionally pick up the wilt organism and carry it internally in

¹³ In a previous publication (9), fall panicum (*Panicum dichotomiflorum* Michx.) was incorrectly referred to as Johnson grass (*Sorghum halepense* (L.) Pers.).

small numbers, but the high proportion of atypical colonies suggests unfavorable environmental conditions for the bacteria in these insects.

CHAETOCNEMA CONFINIS¹⁴

During the 4-year period 1934-37, 238 isolations were made from 1,289 adults of *Chaetocnema confinis* and only 4 isolations of *Aplanobacter stewarti* were obtained. These records were made during 1934 and 1935, when identifications of this species were not so accurately made as in 1936 and 1937, and it seems quite probable that this species was confused with *C. pulicaria* during the early part of the work. From the records obtained it would appear that *C. confinis* is not a vector of *A. stewarti* under field conditions. In feeding tests, however, in which adults were confined to infected corn in cages for several days and then tested by isolation in the usual manner, 48 isolations from 128 adults of *C. confinis* yielded 6 cultures of *A. stewarti* during 1935 and 1936, indicating that this species did pick up the organism and carry it internally, for a short time at least, under the conditions of these tests. On the plates poured from suspensions of *C. confinis*, there were either a few typical colonies of *A. stewarti* or atypical yellow colonies that upon inoculation into corn proved to be *A. stewarti*. Most of the cultures contained nothing resembling the wilt organism. All attempts to inoculate healthy corn by means of adults of this species, that had been confined to infected corn for several days, were unsuccessful.

DIABROTICA DUODECIMPUNCTATA¹⁵

Diabrotica duodecimpunctata has long been considered important as a vector of *Aplanobacter stewarti* in the field, but if one may judge from the data obtained during the 4-year period 1934-37, its importance has been overestimated. The writers have not previously confirmed direct summer dissemination of *A. stewarti* by *D. duodecimpunctata* as reported by Rand and Cash (10, p. 30), but have reported transmission of *A. stewarti* from infected to healthy corn only when the insects were confined in cages (9). Not until 1935 was the wilt organism isolated from field collections of adults of *D. duodecimpunctata*, when 54 isolations, made from 322 field-collected individuals, yielded 3 cultures of *A. stewarti*. During 1934, 20 isolations were made from 178 field-collected adults, which yielded no *A. stewarti*, and during 1936 and 1937, 40 isolations, made from 138 field-collected *D. duodecimpunctata*, yielded no *A. stewarti*. During the 4-year period 1934-37, a total of 114 isolations were made from 638 field-collected *D. duodecimpunctata*, and these yielded only 3 cultures of *A. stewarti*. In addition, 13 isolations made from 14 adults of *D. duodecimpunctata* that had fed on infected corn in cages for several days during 1934 and 1935 yielded no *A. stewarti*. These data indicate that this species is not an important factor in the dissemination of bacterial wilt of corn under field conditions. On the plates poured from broth suspensions of *D. duodecimpunctata*, only a few colonies of *A. stewarti* developed in mixed cultures.

DISONYCHA GLABRATA¹⁶

One isolation from two individuals of *Disonycha glabrata* collected from infected corn at the Arlington farm, August 10, 1934, yielded

¹⁴ Coleoptera, Chrysomelidae.

¹⁵ Coleoptera, Chrysomelidae.

¹⁶ Coleoptera, Chrysomelidae.

Aplanobacter stewarti. Plates poured from broth suspensions of this species showed light seedings of the wilt organism. This insect apparently is rarely found on corn, and these specimens may have been swept from *Amaranthus* sp. present in the corn field. Further studies are necessary to determine its status as a vector of *A. stewarti*.

EPITRIX CUCUMERIS¹⁶

During the 4-year period 1934-37, 121 isolations were made from 1,150 adults of *Epitrix cucumeris*, and 3 isolations of *Aplanobacter stewarti* were obtained during 1935 and 1936. The isolation of *A. stewarti* from this species during 1935 was obtained from 1 adult collected from oats near Burlington, N. J., on May 16. In 1936, 1 isolation of *A. stewarti* was obtained from 10 individuals and 1 from 7 individuals, all collected from sweet corn at Selinsgrove, Pa., May 25. One of the plates poured from adults of *E. cucumeris* had a light seeding of *A. stewarti*, but the other two showed only a few yellow colonies that were not typical.

PHALACRUS SP.¹⁷

During 1936 and 1937, 34 isolations were made from 121 adults determined as *Phalacrus* sp. One culture of *Aplanobacter stewarti* was obtained from a broth suspension of 5 adults collected on corn at Geneva, N. Y., August 28, 1937. The plates showed a heavy seeding of typical colonies of *A. stewarti*. No explanation is offered for an isolation of *A. stewarti* from a smut- and pollen-feeding beetle collected at a point where very little wilt occurred. No further tests have been made with this species.

STILBUS APICALIS¹⁸

During the 4-year period 1934-37, 20 isolations were made from 148 individuals of *Stilbus apicalis* and 2 isolations of *Aplanobacter stewarti* were obtained, one from 2 individuals collected from infected corn at the Arlington farm, September 5, 1934, and the other from 2 individuals collected from alfalfa at the Arlington farm, November 5, 1935. The plates poured from these latter 2 adults of *S. apicalis* gave mixed cultures of various organisms, but 1 atypical yellow colony was transferred which on inoculation into corn produced typical symptoms of bacterial wilt. No *A. stewarti* culture was obtained from 4 isolations made from 16 adults of *S. apicalis* that were confined to infected corn in cages for several days during 1935 and 1936. The above-mentioned isolation of *A. stewarti*, from a second pollen-feeding beetle, was made in the late fall.

STILBUS VIDUUS¹⁹

During the 4-year period 1934-37, 45 isolations were made from 225 adults of *Stilbus viduus* and 1 isolation of *Aplanobacter stewarti* was obtained. This isolation was from 4 individuals collected from alfalfa at the Arlington farm, November 5, 1935. The plates poured from suspensions of 4 adults of *S. viduus* were similar to those already described for *S. apicalis*. As in the case of *S. apicalis*, this isolation of *A. stewarti* from a third pollen-feeding beetle also was made in late fall. In feeding tests to determine whether this insect would pick up the organism and carry it internally for a few hours at least, no culture of *A. stewarti* was obtained from 5 isolations made from

¹⁶ Coleoptera, Chrysomelidae.

¹⁷ Coleoptera, Phalacridae.

¹⁸ Coleoptera, Phalacridae.

35 adults confined to infected corn for several days in cages during 1936.

NABIS FERUS¹⁹

During the period 1934-37, 11 isolations were made from 28 adults of *Nabis ferus*. One isolation of *Aplanobacter stewarti* was obtained from 6 adults collected at the Arlington farm, April 16, 1936. One atypical yellow colony from a mixed culture produced wilt in corn. Seven isolations made from 42 individuals that had been confined to infected corn in cages for several days in 1935 and 1936 yielded no *A. stewarti*. Although *N. ferus* is considered a predatory insect, after it had been starved for a few hours it was observed to puncture succulent corn leaves and suck the juices from them; so it would be entirely possible for this species to carry the wilt organism internally. No explanation is offered for an isolation of *A. stewarti* from this insect collected April 16, before any infected corn plants were available in the field.

ILLINOIA SOLANIFOLII²⁰

Fifty-nine isolations were made from a total of 415 individuals of *Illinoia solanifolii* during 1934 and 1935, and 1 isolation of *Aplanobacter stewarti* was obtained. This record was secured during 1934 from a collection of 48 individuals of this species made from an infected sweet corn plant at Burlington, N. J., on July 17. An atypical yellow colony produced wilt in corn. Additional tests were conducted in 1936, when 25 isolations were made from 250 individuals that had been confined to infected corn in cages for several days. No *A. stewarti* was obtained, and these comparatively meager data would indicate that this species is not an important vector of bacterial wilt of corn under field conditions.

STIRELLUS BICOLOR²¹

During 1934 and 1935, three isolations were made from seven individuals of *Stirellus bicolor*. In 1934 a light seeding of *Aplanobacter stewarti* was obtained from one isolation from four adults collected from infected sweet corn at the Arlington farm, August 10. This species is not commonly found on corn and no further tests were made with it, except that one adult which had been confined to infected corn for several days during 1935 was found to yield no *A. stewarti* when tested in the usual manner.

THAMNOTETRIX NIGRIFRONS²¹

During the period 1934-36, 13 isolations were made from 221 adults of *Thamnotetrix nigrifrons*. In 1934, 1 isolation, made from a collection of 26 adults from infected sweet corn at the Arlington farm, August 10, yielded *Aplanobacter stewarti*. Three typical yellow colonies of *A. stewarti* developed in a plate of white colonies. One isolation made from 9 adults that had been confined to infected corn for several days during 1935 yielded no *A. stewarti*.

ANAPHOTHRIPS OBSCURUS²²

During the 3-year period 1934-36, 23 isolations were made from 603 individuals of *Anaphothrips obscurus*. One isolation of *Aplano-*

¹⁹ Hemiptera, Nabidae.

²⁰ Homoptera, Aphididae.

²¹ Homoptera, Cicadellidae.

²² Thysanoptera, Thripidae.

bacter stewartii was obtained from a collection of 17 individuals (not disinfected externally) found on an infected sweet-corn plant at Hempstead, N. Y., May 29, 1936. The plates showed a heavy seeding of atypical yellow colonies. No feeding tests on infected corn were made with this species, and its importance as a vector of *A. stewartii* under field conditions is undetermined.

ADDITIONAL TESTS

Since *Aplanobacter stewartii* had been isolated from field collections of several species of insects, most of the species discussed earlier in this paper and many others were confined to infected corn in cages for several days and then tested in the usual manner to determine which species might pick up the organism under such conditions. The species used in these tests are included in table 6 and are designated by footnote 3 of that table. In many instances, only a few individuals of a species were used for isolation after feeding on infected corn. It was very difficult to keep infected corn plants in a suitable condition to be fed upon for several days by large numbers of insects confined in cages, and some of the species lived but a short time under these conditions.

Species tested but not included in table 6, were *Graphocephala versuta* (Say), *Hysteronura setariae* (Thos.), *Anthonomus* sp., *Frankliniella williamsi* Hood, and *Peregrinus maidis* (Ashm.). Tests of probable significance were conducted with the following species, the results of which have not been reported earlier in this paper: 9 isolations from *Aphis maidis* made from 142 individuals; 3 isolations from *Agallia constricta* made from 28 individuals; 19 isolations from *H. setariae* made from 410 individuals; 2 isolations from *P. maidis* made from 41 individuals; 3 isolations from *F. williamsi* made from 80 individuals; 3 isolations from *Lygus pratensis oblineatus* (Say) made from 124 individuals. *Aplanobacter stewartii* was not obtained from any of these species.

BACTERIAL WILT ON CORN RELATIVES

Included with *Zea mays* in the tribe Tripsaceae are the closest wild relatives of corn, *Euchlaena*, or teosinte, and *Tripsacum*, or gama-grass. *Zea mays* and *E. mexicana* Schrad. cross freely, and the hybrids are fertile. *E. perennis* Hitchc., the perennial species, crosses less readily with corn, and the hybrid plants are usually sterile.

Euchlaena mexicana, the annual species, has been found (3) to be susceptible to natural infection with bacterial wilt in the field and to inoculation in the greenhouse by means of pure cultures or by feeding of infested corn flea beetles. The Florida form was the one observed and tested. This form has been reported (1) as producing less fertile hybrids with corn than other forms of the annual species. Inoculations of three plants of *E. perennis* in the greenhouse, by introducing pure cultures of *Aplanobacter stewartii* into the leaf and base of the stalk, showed that this species also is susceptible to wilt infection. Young plants, 10 to 12 inches high, developed long water-soaked streaks and general wilting. One plant was dead a week after inoculation. Another, with a long wilt streak the length of one leaf, outgrew the infection. Seven plants of *E. perennis* were grown in the field at Arlington farm next to heavily infected sweet corn, during the 1937

season. Local leaf lesions developed from natural infection on all of these plants.

Tripsacum is the next closest but much more distant relative of corn (2). Crosses with corn have only recently been made by using corn as the female plant, and the plants were sterile. Hitchcock (6) lists three species of *Tripsacum* as native to the United States. Two of these, *T. dactyloides* (L.) L. and *T. lanceolatum* Rupr. have been tested for susceptibility to wilt along with two other species with broader leaves, *T. pilosum* (Scribn. and Merr.) from Amparo, Mexico, and *T. latifolium* Hitchc. from Honduras.²⁴ *T. dactyloides* is widely distributed over the eastern part of the United States and grows wild at the Arlington farm and in other nearby places. No bacterial wilt lesions have ever been found on any of these wild plants on or near the Arlington farm, although they were under observation for 4 years 1934-37.²⁵ Eighteen field inoculations of young growing shoots of these plants failed to produce any wilt symptoms. No wilt symptoms developed on either the Kansas or Maryland forms of *T. dactyloides* from six inoculations of each in the greenhouse. Nor did any lesions develop on *T. dactyloides* in 1935 after apparently infested adults of *Chaetocnema pulicaria* confined in cages in the greenhouse had fed on the leaves. Seven inoculations each of young shoots and leaves of *T. lanceolatum*, *T. latifolium*, and *T. pilosum* in the greenhouse failed to produce any wilt symptoms. During the 1936 and 1937 growing seasons two plants each of *T. lanceolatum*, *T. latifolium*, and *T. pilosum* were grown at the Arlington farm next to plots of infected sweet corn. These grew into large spreading plants 5 to 6 feet tall with many shoots and leaves. During both seasons *C. pulicaria* was observed feeding on all three species of *Tripsacum*, and old feeding injuries were numerous. No wilt lesions could be found, however, on any of these plants, and no wilt developed from eight inoculations made on young leaves and shoots of each species in 1936. The hybrid *Tripsacum dactyloides* × *Zea mays* and plants of the corn parent have been tested by inoculation in the greenhouse. The corn parent was moderately susceptible to wilt. Typical wilt lesions developed throughout the length of the inoculated leaves of six plants. These leaves died, but the plants continued to grow normally without further evidence of wilt, although pure cultures of the wilt organism had been introduced into the stem. Ten plants of the corn parent were grown in the field at the Arlington farm beside infected sweet corn during the 1937 season. All of these plants developed local wilt lesions on the outer halves of the leaves. Inoculations in the greenhouse of the one hybrid plant available produced no wilt symptoms. On June 10, 1937, this hybrid was planted in the field beside susceptible sweet corn. Insect-feeding injuries were observed during the season, and by the middle of September reddish-brown streaks one-fourth of an inch long on the leaves were found to contain abundant bacteria. Three isolations from these lesions gave typical colonies of *Aplanobacter stewarti*, which, on inoculation, produced wilt in susceptible sweet corn in the greenhouse. These lesions had apparently developed from insect-feeding injuries and did not increase further in size.

²⁴ Plants furnished by J. H. Kempton, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

²⁵ *Tripsacum dactyloides* was later reported to be a native host of *Aplanobacter stewarti* by Elliott and Robert (6).

Three Old World genera—*Coix*, *Sclerachne*, and *Polytoca*—belonging to the tribe Tripsaceae have been tested in the greenhouse and field. *Coix lacryma-jobi* L. has previously been reported as a host for *Aplanobacter stewarti* when inoculated in the greenhouse (9). During the summer of 1935, 4 plants each of 3 varieties of *C. lacryma-jobi*,²⁶ namely, Lackfoo, Glutinous, and wild, were grown in the field at the Arlington farm next to infected sweet corn. No inoculations were made, but *Chaetocnema pulicaria* fed on the leaves throughout the season and typical long wilt streaks developed. During the 1936 season 4 plants each of *Sclerachne punctata* R. Br. and *Polytoca barbata* Stapf²⁷ and, in 1937, 36 plants of *S. punctata*²⁸ were grown at the Arlington farm next to heavily infected sweet corn. Individuals of *C. pulicaria* were observed feeding on these plants, and although feeding injuries were abundant on the leaves no wilt lesions developed and no bacteria could be found in or around the feeding injuries. Ten inoculations were made on young leaves and shoots of each species in 1936. The only evidence of any infection was a reddening of the veins of an inoculated leaf of *Sclerachne* for several inches beyond the leaf injury. Two inches below the point of inoculation the veins were full of bacteria. *A. stewarti* was reisolated and produced wilt on corn. The lesion was slight, however, and the infection did not progress further. Later in 1936, 2 sets of 4 inoculations each were made on young shoots of *Polytoca* and *Sclerachne* in the greenhouse. No wilt symptoms developed on the young shoots of the former. The inoculated shoots and leaves of *Sclerachne* dried and died. The leaf veins and fibrovascular bundles of the stalk were full of bacteria, and *A. stewarti* was reisolated from both. As stated above, no natural infection developed in the field the following summer.

The next tribe, the Andropogoneae, is not widely separated from the Tripsaceae (2). The various forms of maize completely bridge the gap between the two tribes. Two genera of considerable economic importance, *Saccharum officinarum* L. and *Sorghum vulgare* Pers. (*Holcus sorghum* L.) have been tested for susceptibility to wilt by the senior author and by other investigators.

In 1935 Ivanoff (8) reported that inoculations in the greenhouse on *Sorghum vulgare*, varieties Waconia (Rox) Orange sorgo, Blackhull kafir, darso, Dwarf Yellow milo, Kansas Orange sorgo, Gooseneck sorgo, and Red Amber sorgo, produced reddening of infected veins and in some cases yellowing of the leaf tissue outside the red areas. *Aplanobacter stewarti* was reisolated from these lesions. No leaf symptoms resulted from 2 inoculations in the field of over 1,000 plants of Waconia Orange sorgo and Kansas Orange sorgo. Red bundles appeared in the stalks of Waconia Orange but not in Kansas Orange. In a series of field inoculations on 300 Sudan grass plants, "only a small percentage of the plants showed any symptoms" and "these symptoms were not considered striking." In another set of inoculations symptoms were similar to those on corn. Ivanoff was not able to produce any infection in 25 inoculated sugarcane plants.

Similar tests for susceptibility to bacterial wilt have been carried on by the senior author in the greenhouse and in the field at the

²⁶ Seed from the Plant Pathology Laboratory, Bureau of Plant Industry, Manila, P. I.

²⁷ Seed from Dr. Paul Weatherwax. Grown on campus of Indiana University, 1935; original seeds from Poona, India.

²⁸ Seed from Dr. Paul Weatherwax, Indiana University. Grown at Bloomington, Ind., 1935; original seed from Buitenzorg, Java.

Arlington farm. The following genera and species were inoculated in the greenhouse: *Sorghum halepense* (L.) Pers. (Johnson grass); *S. vulgare sudanense* (Piper) Hitchc. (Sudan grass); certain commercial varieties of *S. vulgare*, namely, White durra, hegari, Blackhull kafir, Dawn kafir, Blackhull kaoliang, Manchu Brown kaoliang, Standard Yellow milo, Dwarf White milo, Dwarf Yellow milo, Colman sorgo, Dakota Amber sorgo, Folger sorgo, Orange sorgo, Sumac sorgo, Black Amber sorgo, shallu, Spur feterita, Leoti Red sorgo, Fargo Straight-neck milo, Evergreen Standard broomcorn, and darso; and 13 cultivated varieties of *Saccharum officinarum*,²⁹ namely, D-74,³⁰ Louisiana Purple, C. P. 807, C. P. 29-291, C. P. 28-19, C. P. 29-320, C. P. 29-116, Co. 290, Co. 281, P. O. J. 36M, P. O. J. 234, P. O. J. 213, and Uba.

No infection developed on Johnson grass or Sudan grass. All of the inoculated sorghum plants were stunted as compared with uninoculated checks, probably owing to injury. In 7 of the varieties the central shoot was killed and side shoots developed. In 8 of the varieties reddening of injured veins developed, and in 4 cases there was slight yellowing of the tissue along these veins. Isolations from such lesions on hegari gave yellow colonies which did not produce wilt in sweet corn. Isolations from red veins of 11 other varieties of sorghum gave negative results. There was also some reddening of bundles of the stalk, mostly in the inoculated internode. The sorghum plants all outgrew the effects of inoculation and produced heads, whereas the susceptible corn plants succumbed to the disease. On sugarcane slight yellowing of leaf veins developed on some of the plants, but the plants soon outgrew any symptoms of injury and bacteria were not found in the veins except about the point of inoculation.

With the exception of the sugarcane varieties, 6-foot rows of 12 plants each of the foregoing genera, species, and varieties of corn relatives were planted in the field at the Arlington farm early in June 1935, in the same plot with sweet corn, which during the season was destroyed by wilt. Inoculations of 6 plants of each variety were made in the same way as in the greenhouse, and in addition notes were taken on insect-feeding injuries and natural infection. No infections resulted from inoculations on Johnson grass. In some of the sorghums reddening of the veins and yellowing of tissue along the veins developed for an inch or two beyond the inoculation injuries; but no bacteria were isolated, the lesions did not develop further, and the plants grew normally. Corn flea beetles were abundant during the 1935 season and were observed feeding to some extent on all of the varieties tested. Throughout the season, however, no signs of wilt appeared on any of the plants. On *Euchlaena* and *Coix*, growing nearby, typical long wilt streaks developed. Bacteria were abundant in the lesions and *Aplanobacter stewarti* was isolated from both genera.

Manisuris cylindrica (Michx.) Kuntze, another of the Andropogoneae, developed no wilt symptoms from inoculations in the greenhouse or from 12 inoculations in the field. In 1936, 12 large plants and in 1937, 2 clumps of plants grew next to heavily infected sweet

²⁹ Plants supplied by R. D. Rands and E. Dopp, of the Division of Sugar Plant Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

³⁰ The letters designating the sugarcane varieties indicate experiment stations where the varieties were developed: D = Demerara; C. P. = Canal Point, Barbados; Co. = Colmbatore; P. O. J. = Proef station Ost, Java.

corn in the field. Individuals of *Chaetocnema pulicaria* were observed feeding on the leaves, and feeding injuries were present on the outer halves of many leaves; but no natural infections developed and no bacteria could be found in or around the insect-feeding injuries.

Setaria glauca, in the Paniceae, the next tribe of the Gramineae, has been reported by Ivanoff (7) as developing white or tan stripes after inoculation in the greenhouse. Six field and four greenhouse inoculations of this species at the Arlington farm did not produce bacterial wilt symptoms.

The results of the inoculations at Arlington farm indicate that yellow bristle grass (*Setaria lutescens*), Johnson grass, Sudan grass, sugarcane, *Manisuris cylindrica*, *Tripsacum* species, *Polytoca*, and probably *Sclerachne* are not natural hosts of *Aplanobacter stewarti*, although slight reddening or yellowing of fibrovascular bundles may result from greenhouse inoculations. In the opinion of the senior author plants that show no wilt symptoms when inoculated in the field or when subjected to feeding by *Chaetocnema pulicaria* can scarcely be considered as host plants of *A. stewarti*.

Species and varieties of *Coix* and *Euchlaena* are the only ones found up to the present time at the Arlington farm that have developed typical wilt symptoms from artificial as well as natural inoculation. Of the native American genera and species, *Euchlaena mexicana*, the closest relative of corn, is the one most susceptible to infection. *E. perennis*, less closely related to corn, is somewhat less susceptible. *Tripsacum* species, the next closest relatives, have been immune to wilt in these experiments.

SUMMARY

At the Arlington Experiment Farm, near Washington, D. C., in 1934, 1935, and 1937, bacterial wilt was abundant and destructive. Sixty to eighty percent of the susceptible Golden Bantam sweet corn plants became infected in the early leaf stage, and as a result many of the plants were stunted or killed and relatively few marketable ears were harvested. The wilt situation in 1936 was quite different. There was very little infection in the early leaf stage, and although infection became general as the season advanced most of the lesions did not develop beyond local leaf infections. The time for material injury to the sweet corn crop had passed, and a good crop of marketable ears was harvested.

The mean temperature for December, January, and February, 1933-34, at Washington, D. C., was slightly below the mean normal of 35.1°, but well above 32° F. In 1934-35 the mean winter temperature was slightly above normal. In 1935-36 the winter was much colder, the average temperature falling slightly below 32°. In 1936-37 the average was above 40°.

At the Arlington farm in 1934, 1935, and 1937 adults of *Chaetocnema pulicaria* were abundant early in the season on young corn plants. In 1936, these beetles were much less abundant in May and June. By July 15 they showed a noticeable increase in numbers, and by August 15 they were abundant on the corn plants. In 1937, at the Arlington farm, adults of *C. pulicaria* again were abundant on corn early in the season.

At Geneva, N. Y., not a single wilt lesion was found in 1936, but in 1937, 11 percent of the susceptible Golden Bantam plants were

infected. In 1936 mean winter temperatures at Geneva were below 32° F., whereas in 1937 they were well above 32°. Upon three occasions (when weather conditions were not most favorable for collecting this species), no specimens of *Chaetocnema pulicaria* were found at Geneva in 1936, but late in August some evidence of feeding by this insect was observed, indicating that this species was present in small numbers at this time. Fifty-seven specimens of *C. pulicaria* were collected at Geneva in late August 1937, and 18 percent of these were found to be infested with *Aplanobacter stewarti*.

At Yonkers, N. Y., in 1935, 9 percent of the early planting of susceptible Golden Bantam plants were infected and 3 percent of these were killed. In 1936 no wilt was found by the middle of June on 700 Golden Bantam plants. By the middle of August, 4 percent were found infected. In 1937, 5 percent were infected early in the growth of the plants and 99 percent when the plants were mature or in silk.

On Long Island in 1936 there was little wilt early in the season but abundant wilt by the middle of August. Wilt records for 1937 show more early infection than in 1936. In New York City mean winter temperatures for 1936 were below 30° F. and for 1937 above 38°.

During the 4-year period 1934-37, 28,769 insects representing 94 species belonging to 76 genera, were tested for *Aplanobacter stewarti* to determine which species, and what proportion of each, carry the wilt organism internally and may be vectors of bacterial wilt under field conditions. Results obtained by testing 18,613 individuals of *Chaetocnema pulicaria* during the 4 years indicate that it is the only species of importance in harboring the wilt organism over winter and also in spreading the disease during the corn growing season.

Chaetocnema denticulata may possibly play some part in wilt dissemination, but at the Arlington farm it is not so abundant as *C. pulicaria* and it feeds preferably on panic grass. While *Aplanobacter stewarti* sometimes occurs in abundance in *C. denticulata*, which ranks next to *C. pulicaria* in numbers of adults from which *A. stewarti* has been isolated, *C. denticulata* is apparently not a close second in importance to *C. pulicaria* and it is doubtful whether the wilt organism ever overwinters in adults of *C. denticulata*.

From results obtained, none of the other species from which *Aplanobacter stewarti* has been isolated can be considered vectors of any importance under field conditions. It seems to be quite possible for insects that occasionally feed on corn to pick up *A. stewarti* from infected plants, but judging from the type of cultures obtained, these insects do not furnish a favorable environment for the bacteria and are apparently of no importance in the spread of bacterial wilt.

In the species of *Chaetocnema* the proportion of infested beetles has usually been greater when the insects were caged on infected corn than when they were collected from the field. In 1934, *Aplanobacter stewarti* was isolated from 56 to 68 percent of the *Chaetocnema pulicaria* individuals caged on infected corn and in 1935 from 60 to 95 percent: in 1934, it was isolated from 48 percent of the caged individuals of *C. denticulata* and in 1935 from 61 percent.

Further proof of the overwintering of *Aplanobacter stewarti* in hibernating adults of *Chaetocnema pulicaria* was obtained. Seven beetles collected March 16, 1936, were fed on corn plants in the greenhouse, and typical wilt symptoms developed. *A. stewarti* was isolated from *C. pulicaria* collected at the Arlington farm in every

month of the year except February during the 4-year period, and one isolation was made from beetles collected February 26, 1936, at Norfolk, Va.

Early- and late-season isolations from *Chaetocnema pulicaria* from other localities have shown that overwintering adults of *C. pulicaria* are more or less infested with *Aplanobacter stewarti* in all sections of the country where wilt is prevalent and isolations have been made.

In studies of the host range of *Aplanobacter stewarti*, inoculations in the greenhouse on rapidly growing young plants may cause stunting and some discoloration along the veins. If, however, such lesions or discolorations do not progress and if new shoots can develop and grow normally without further evidence of infection, such plants should be tested further by being subjected to natural infection in the field. If these plants, growing under natural conditions in the field next to heavily infected sweet corn and injured by feeding of adults of *Chaetocnema pulicaria* which were spreading the infection on corn and many of which were undoubtedly infested, show no signs of bacterial wilt they can scarcely be considered hosts for the bacterial wilt organism. *Euchlaena perennis*, in addition to *E. mexicana* and *Coix lacryma-jobi* already reported, was found to be susceptible to infection with the wilt organism. *Tripsacum dactyloides*, *T. pilosum*, *T. lanceolatum*, and *T. latifolium* were immune to wilt in these experiments. Other genera and species tested did not develop typical wilt symptoms.

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GENETIC AND CYTOLOGIC STUDIES OF A BRACHYTIC MUTATION IN BARLEY¹

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INTRODUCTION

Studies of the action of genes from the time they enter the gametes until they produce their final effects on the characters of mature individuals are still in an elementary state of development. Genes having a marked effect on size characters furnish excellent material for studies of this nature, especially if a single gene may be studied by itself.

In 1929 genetic studies were begun at the Minnesota Agricultural Experiment Station on a dwarf barley which had arisen spontaneously as a mutation in a plot of Himalaya barley grown by Dr. L. J. Stadler at the Missouri Agricultural Experiment Station. Dr. F. J. Stevenson, then in charge of barley genetics at the Minnesota station, began these studies for the purpose of determining the linkage relationships of the gene producing the dwarfed condition.

In correspondence with Stevenson, Stadler described the mutation as follows: "A shortened plant with short leaves, short awns, and short internodes, something like brachytic corn but far less extreme. It is distinct enough to be easily separated, and equal in viability to the normal." Aside from its shortened appearance, the mutation is very similar to Himalaya, which is a six-rowed, rough-awned, and hullless variety, grown commercially to some extent in the western part of the United States. The new dwarf was given the name Brachytic, and the notation *br* has been used by Powers (28)³ for the gene. A comparison of the general appearance of Brachytic and Himalaya is shown in figure 1.

The studies were continued later by Powers (28), who reported on the mode of inheritance of the brachytic character. The studies reported in this paper on the cytological and histological nature of the brachytic character were suggested by Powers in 1932, and the data on the linkage relationships of the brachytic gene were turned over to the writer by Powers in 1935.

The following considerations are presented in this paper: (1) Further studies on the inheritance of the brachytic habit of growth; (2) the linkage relationships of the gene for brachytic; (3) the cytological nature of the mutation, whether it is the result of a gene

¹ Received for publication June 3, 1939. Presented to the faculty of the graduate school of the University of Minnesota in partial fulfillment of the requirements for the degree of doctor of philosophy. Paper No. 1714 of the Journal Series, Minnesota Agricultural Experiment Station.

² The writer wishes to express his sincere appreciation to Dr. F. R. Immer for kindly criticisms and helpful suggestions during the preparation of the manuscript and to Dr. L. R. Powers for suggestions and contributions of material during the course of the experiment.

³ Italic numbers in parentheses refer to Literature Cited, p. 711.



FIGURE 1.—Comparison of plants of Himalaya (A) and Brachytic (B).

mutation or a chromosomal change; and (4) the nature of the action of the brachytic gene with respect to plant and organ size, cell size and number, growth rate, and frequency of cell division.

REVIEW OF LITERATURE

THE INHERITANCE OF SIZE CHARACTERS

The classic work on genes affecting plant size is that of Keeble and Pellew (15), who showed that two factors were involved in differentiating between a short and a very tall race of peas. In general, size characters have been found to be affected by several or a large number of factors, according to the multiple-factor hypothesis advanced by Nilsson-Ehle (26) and East (5). However, a few size characters, determined by only one or a few genes, have been studied in plants.

Linkage between factors for quantitative or size characters and factors for qualitative characters has been demonstrated by Sax (32) and Sirks (38) in beans, Lindstrom (20) in tomatoes, and Kempton (16, 17) in maize. The character studied by Kempton was a brachytic variation, similar in behavior to the brachytic character in barley which is being discussed in this paper. Similar types of linkage in barley have been reported by Griffie (11), Neatby (25), Wexelson (43, 44), and Powers (28), and suggested by Robertson and Austin (30).

LINKAGE RELATIONSHIPS IN BARLEY

The literature on linkage relationships in barley has been reviewed extensively in a recent paper by Robertson (29). It therefore seems unnecessary to review it further than to state that, according to Robertson, all of the expected seven linkage groups have been established and that three or more factors have been located in each group. Powers (28) showed that the factor pair for normal vs. brachytic habit of growth (*Br br*) was independent of the factor pairs for non-six-rowed vs. six-rowed spike (*V v*) and black vs. white lemma and pericarp (*B b*) in the first and second linkage groups, respectively.

MEIOSIS AND CHROMOSOME NUMBER IN BARLEY

As far as the writer is aware, no detailed studies have been made of meiosis in barley. Griffiee (11) found that the somatic chromosome number was 14 in cultivated barleys. His counts have been substantiated by Griffiee (12) and Aase and Powers (1).

CELL SIZE IN RELATION TO PLANT AND ORGAN SIZE

The literature pertaining to the relationship of cell size to organ and body size in both plants and animals has been reviewed in detail by Bailey and Tupper (2). Sinnott (36) also has presented a comprehensive review of studies on cell size and shape in plants.

The relationship of cell size to dwarfism or nanism has been investigated by Gauchery (7) and Sierp (35). Gauchery concluded that dwarfs often have smaller cells but that different tissues react very differently to conditions inducing dwarfism. Sierp called attention to the necessity of distinguishing between depauperate plants and true constitutional dwarfs. The former have smaller cells than normal. He recognized three classes of inherited dwarfing: dwarfs of *Pisum*, *Solanum*, *Zea*, and *Clarkia*, having smaller cells than normal; *Nigella*, larger than normal; and *Mirabilis* and *Lathyrus*, normal.

GROWTH RATES AND GENETIC FACTORS

According to Sinnott and Dunn (37), differences in size may be correlated with differences in rate of growth, length of the growth period, or both. Goldschmidt (9) has postulated factors which determine the rate at which developmental processes occur and has recently (10) discussed this subject in more detail.

MATERIALS AND METHODS

INHERITANCE OF THE BRACHYTIC CHARACTER

Data on the inheritance of the brachytic character were taken from all the crosses used in determining the linkage relationships of the brachytic gene. A small F_2 population of Brachytic \times Himalaya also was studied.

LINKAGE RELATIONSHIPS OF THE GENE FOR BRACHYTIC (*br*)

Six varieties and strains were crossed with Brachytic in order to test for linkage of the brachytic gene in all of the seven linkage groups. These varieties and strains with the characters studied and the years and generations in which the studies were made are presented in

table 1. With respect to the characters used, the genotype of Brachytic then would be *vv bb nn kk OO X_c X_c F_c F_c*.

The crosses with B1, B3, B4, and Orange Lemma had been made in 1930 and 1931 by F. J. Stevenson and L. R. Powers, and were classified and studied by the latter in the F₂ generation with the aid of the writer. The cross with B1 was subjected to an F₃ progeny test by Powers in 1934. The crosses with Colseess IV and Colseess V, obtained from D. W. Robertson of the Colorado Agricultural Experiment Station, were made in the greenhouse in 1933, and the F₁ generation was grown in the field during the summer of the same year. When an association was found in 1934 between the brachytic gene (*br*) and the dominant normal allele (*F_c*) of the chlorina gene in Colseess V, 130 F₂ plants of this cross were saved for an F₃ population in 1935 on the basis of the genotype exhibited in the F₂ generation.

TABLE 1.—*Contrasting characters and varieties or strains used to test for the linkage relationships of the gene for brachytic (br)*

Contrasting character	Symbol	Linkage group	Variety or strain	Generation and year
Non-six-rowed spike	V	I	{B1..... B3..... B4.....	F ₂ , 1932; F ₃ , 1934. ¹ F ₂ , 1932. ² F ₂ , 1931 and 1933.
Black lemma and pericarp	B	II	{B1..... B3..... B4.....	F ₂ , 1932; F ₃ , 1934. ¹ F ₂ , 1932. ² F ₂ , 1931 and 1933.
Covered caryopsis	N	III	{B1..... B3..... B4.....	F ₂ , 1932. ² F ₂ , 1931 and 1933.
Hooded lemma	K	IV	{Orange lemma..... B3.....	F ₂ , 1932. ² F ₂ , 1932.
Orange lemma	o	V	{Orange lemma..... Colseess IV (heterozygous).....	F ₂ , 1932. F ₂ , 1934.
Xantha seedlings	x	VI	{Colseess IV (heterozygous)..... Colseess V.....	F ₂ , 1934. F ₂ , 1934; F ₃ , 1935.
Chlorina seedlings	f _c	VII		

¹ Reported by Powers (23).

² From unpublished data of Powers and Swenson.

All characters except xantha and chlorina seedlings were classifiable in the laboratory. Xantha seedlings were counted as soon as they emerged since they died soon after emergence. Chlorina seedlings were marked in the field by placing stakes close to the seedlings when they emerged since the chlorina character is often difficult to classify when the plants become more mature. In the crosses of Colseess IV and Colseess V with Brachytic, the classification of the brachytic character also was made in the field.

COMPARISON OF CHROMOSOME BEHAVIOR AND NUMBER IN BRACHYTIC AND NORMAL STRAINS OF BARLEY

Three F₁ plants from the cross B4 × Brachytic and one F₁ plant from the cross Brachytic × Himalaya were available for cytologic studies in 1932 and 1934 respectively. Spikes with anthers containing the various stages of meiosis were killed in acetic alcohol and transferred after 12 hours to 70 percent alcohol, according to the method outlined by McClintock (21). Preparations were made by the acetocarmine smear method as described by McClintock (22, 23).

In 1932 seed from 24 F₂ plants of B4 × Brachytic and from the parents was space-planted in 6-foot rows to facilitate individual plant studies. Cytological material was taken at random from one row of each parent and from three different F₃ lines—homozygous normal, homozygous brachytic, and heterozygous normal × brachytic. Individual florets with anthers containing the various stages of meiosis

were isolated, special attention being paid to the quartet stage. The florets were killed and fixed in Allen's modification of Bouin's solution and embedded in paraffin blocks according to the schedule outlined by LaCour (18). Sections were cut $12.5\ \mu$. thick and stained by Newton's gentian-violet method. Several florets from the same plant were embedded in the same block and oriented so longitudinal sections of the anther could be made. This provided a larger amount of material in side view which facilitates the examination and counting of the young microspores (27).

Seed from 1932 plants selected on the basis of high and low frequency of micronuclei was planted in 1933 and cytological material taken as in 1932. Similarly, in 1934 high and low selections from 1933 were grown, but instead of embedding and cutting, entire spikes were killed in acetic alcohol and transferred to 70-percent alcohol, later to be studied by the smear method. Himalaya from seed furnished by Stadler was added to the study for comparison with Brachytic in 1933.

The counts of micronuclei were in nearly all cases made on immature microspores grouped in quartets. In a few plants, however, the microspores had broken apart, but since the nuclear material stained well, they could be studied quite readily. Microspores through which the knife had passed were ignored and only micronuclei embedded in cytoplasm were included in the counts. One thousand microspores were counted in each plant.

COMPARISON OF HIMALAYA AND BRACHYTIC

Himalaya, the variety from which Brachytic arose, has been used in all the comparisons made for the purpose of determining the nature of the effect of the brachytic gene on the brachytic character. In so doing, it has been possible to compare Brachytic directly with its progenitor.

Gross morphological characters.—Twenty-five plants of each strain, grown under comparable conditions in 1933, were selected for comparison of length of awns, weight of seed, number of seeds, and weight per seed. Five plants of each strain were selected for comparisons of height of plants, distance between ligules, and length of leaves.

Size of plants, organs, and cells in plants growing in the field.—In 1933, two pairs of comparable plants of Himalaya and Brachytic were collected on June 14 and June 20, respectively, and preserved in the formalin acetic alcohol solution described by Chamberlain (4, p. 21). Measurements were made of total length of different parts, number and length of epidermal cells in different parts, and number and length of parenchyma cells in the culm (fig. 2, A, B, C, D). By dividing the total length of a part by the total number of cells from end to end, it was possible to ascertain the mean length of the cells. This method also provided a more or less absolute comparison of the total number of cells in plants of the two varieties.

Pairs of comparable leaves from Himalaya and Brachytic were collected from time to time and preserved in formalin acetic alcohol. Eight pairs of these leaves were later measured for length and width of blade and for length and width of upper epidermal cells (fig. 2, C).

Leaf width was determined by taking measurements at three different points along the leaf; the first just above the ligule, the second at the widest point of the leaf, and the last about 1 cm. from the tip. These points have been designated as the basal, middle, and terminal

regions, respectively. Cells in the stomatal zones were the most clearly defined and therefore were selected for measurement (fig. 2, *C*). Cell length was determined by counting the number of cells involved in spanning the diameter of a microscopic field provided by a $10\times$ objective with 12.5 oculars and converting to μ by dividing the diameter of the field by the number of cells. Three determinations were made at each of the three points where leaf width was measured, the mean of the three serving as the final value for each point. Cell width was obtained from the mean width of 10 cells in the stomatal

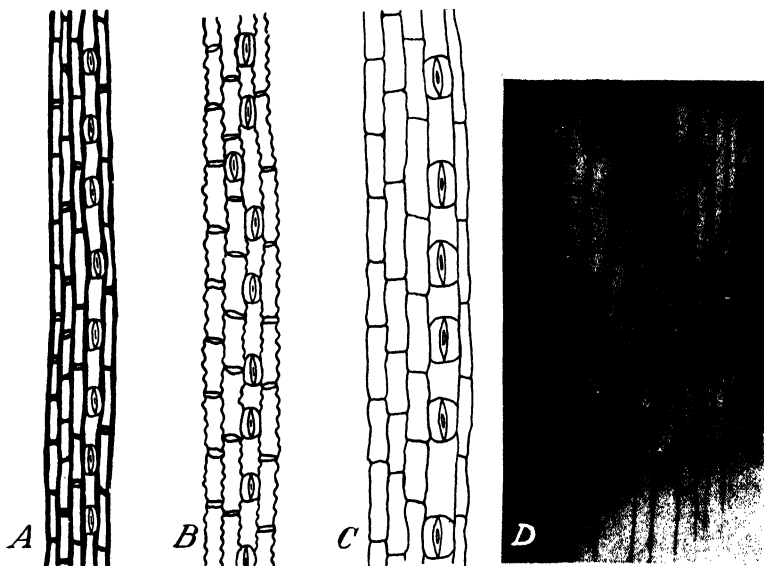


FIGURE 2.—*A, B, C*, Epidermal cells in the internode, leaf sheath, and upper epidermis of the leaf respectively, $\times 75$; *D*, parenchyma cells in the internode of the culm, $\times 150$.

zones at each of the three points, as measured by the eyepiece micrometer.

Staining was found to be unnecessary in making the above studies. Keeping the material soaked on the slide with 50-percent alcohol rendered it satisfactory for clear definition of the cells.

Size of organs and cells in the plumules and primary roots.—Seeds of Himalaya and Brachytic were arranged on a blotter as shown in figure 3 and allowed to germinate in a germinating oven held at 70° F. Beginning on the second day, material was taken daily on 5 successive days from different rows on the blotter. This provided 3 plants of each variety on each day. On the second day, only primary roots were available, but from the third day on, plumules also could be taken from the same plants. Length measurements were made and segments about 8 mm. long were cut from the middle and terminal regions of the root and from the basal, middle, and terminal regions of the plumule. The segments from the basal and middle regions of the plumule provided material from both the coleoptile and primary leaf whereas the terminal segment included only the primary leaf.

The segments were killed and fixed in Navaschin's fluid (18) and cut into paraffin sections $10\ \mu$ thick. Satisfactory staining of both the chromatin material and the cell walls was accomplished by using gentian violet followed by a 1-percent aqueous solution of Congo red

ROW	COLUMN		
I	H : B	H : B	H : B
II	B : H	B : H	B : H
III	H : B	H : B	H : B
IV	B : H	B : H	B : H
V	H : B	H : B	H : B
VI	B : H	B : H	B : H

FIGURE 3.—Arrangement of seed on the blotter for comparing length of plumule and primary root in relation to cell size and frequency of mitosis in Himalaya (H) and Brachytic (B).

Measurements of cell length, taken as in the leaves, were made in the coleoptiles and primary leaves of 12 plants of each variety (fig. 4, *A*, *B*). This represents 4 different rows (dates), 3 plants of each variety per row. For the coleoptile a $10\times$ objective was used, whereas in



FIGURE 4.—*A*, Parenchyma cells in the coleoptile and primary leaf, $\times 125$; *B*, parenchyma cells in the primary leaf, $\times 1,125$.

the primary leaf, which has much smaller cells, a $90\times$ oil-immersion objective was found most suitable. The mean cell length from 10 sections in each of the regions was used as the final value for the region.

Nine plants representing three different rows (dates) for each variety were used in studies of the primary roots. Cell width was determined

by measuring the width of the root in longitudinal section and dividing by the number of rows of cells across the root. Means based on five sections from each region were used as final values. Cell length in the middle region was obtained as in the coleoptile except that only five sections were used. In the root tip a cell-number gradient was obtained by counting the number of cells spanning the microscopic field beginning at the tip and progressing back, 1 field at a time for a distance of 12 fields. Three such determinations were made in each of the five sections per plant with a $45\times$ objective and 12.5 oculars, the mean of the 15 determinations serving as the final value of each plant.

Daily growth increments.—The five plants used for the comparison of gross morphological characters of Himalaya and Brachytic also were measured for the purpose of determining the relative daily growth increments of the two strains. Measurements of height up to the tip of the upper leaf were taken at 2- to 6-day intervals from May 20 to June 20.

Frequency of mitosis.—The root tips were studied further for frequency of mitosis in the meristematic region. All stages of mitosis were counted in the five sections of each plant where the cell number gradient was determined. Length of the meristematic region was calculated from the number of microscopic fields traversed to include its length whereas width was measured directly with the eyepiece micrometer. It was therefore possible to calculate the approximate area and determine the number of mitoses per section and per square millimeter.

STATISTICAL METHODS

Tests for goodness of fit of observed to theoretical ratios were made by the use of χ^2 for goodness of fit. χ^2 for independence was used for testing for independence or association between the factor pair for normal versus brachytic and the factor pairs employed as testers in the different linkage groups. The corresponding P values were obtained from Fisher's (6) table of χ^2 .

The formulae for calculating linkage intensities were obtained from the publications of Immer (13, 14). The F_3 population of Colse V \times Brachytic was selected in such a manner that the method outlined by Immer (14) might be applied to the data.

Fisher's (6) t and Snedecor's (39) F tests were used throughout for testing the significance of differences between Brachytic and the normal strains with which it has been compared.

EXPERIMENTAL RESULTS

INHERITANCE OF THE BRACHYTIC CHARACTER

The ratio of normal to brachytic plants is presented in table 2 as a total for the two classes from the F_2 segregations of all the crosses except the F_2 of Colse V \times Brachytic. This cross has been listed separately because of the deficiency of normal types, later shown to be the result of a deficiency of chlorina types among the normal plants. The brachytic character is undoubtedly inherited as a simple Mendelian recessive.

TABLE 2.—Ratio of normal to brachytic plants in all crosses between brachytic and normal strains

Cross	Phenotype		χ^2 for goodness of fit to a 3:1 ratio	P
	Normal	Brachytic		
All crosses except F_2 of Colless V \times Brachytic	11,282	3,698	0.786	0.50-0.30 (¹)
Colless V \times Brachytic (F_2)	1,804	832	60.554	

¹ Very small.

LINKAGE RELATIONSHIPS OF THE GENE FOR BRACHYTIC

INTERRELATIONSHIPS BETWEEN THE *Br br* FACTOR PAIR AND FACTOR PAIRS LOCATED IN THE DIFFERENT LINKAGE GROUPS

In the first five linkage groups, it was found by applying χ^2 tests for independence that the *Br br* factor pair was inherited independently of all the testers in these groups.

In the cross Colless IV \times Brachytic, it was impossible to identify the normal and brachytic plants in the $x_c x_c$ class. Consequently the xantha plants were grouped and the ratio obtained was compared with a theoretical 9 : 3 : 4 ratio. The ratio obtained was 1,381 *Br X_c* : 469 *br X_c* : 476 $-x_c$ (*Br x_c* or *br x_c*) as compared to the expected ratio of 1,308.37 : 436.13 : 581.50, giving a χ^2 value of 25.649 and a *P* value of less than 0.01. It was noted in taking the data on seedlings, however, that the xantha seedlings were very weak and that many of them might have failed to emerge. Consequently, a deficiency in the xantha class might be expected. When the first two classes were tested to a 3 : 1 ratio, a χ^2 value of 0.122 with a corresponding *P* value of 0.80-0.70 was obtained, indicating that these two classes were occurring in the proportions expected on the basis of independent inheritance.

Chlorina (*f_c f_c*) plants usually survive and produce seed, but are considerably weaker than normal green plants. Furthermore, it is possible to distinguish between the normal and brachytic phenotypes within the chlorina class as shown in figures 5 and 6. The results from the cross between Colless V and Brachytic are presented in table 3. The X^2 values obtained strongly suggest linkage between *Br br* and *F_c f_c* in the seventh linkage group.

ESTIMATING THE LINKAGE INTENSITY BETWEEN *Br br* AND *F_c f_c*

The cross-over value from the F_2 data was calculated by the product method, Immer's tables (13) being used. Substituting the values from the F_2 in table 3 in the formula for the product method, a cross-over value of 30.8 ± 1.8 percent was obtained.

TABLE 3.— F_2 segregation from the cross of normal chlorina (*Br Br f_c f_c*) \times brachytic green (*br br F_c F_c*)

Linkage group	Cross	F_2 ratio				χ^2 for independence	
		<i>Br F_c</i>	<i>Br f_c</i>	<i>br F_c</i>	<i>br f_c</i>	χ^2	<i>P</i>
VII	{ Colless V \times Brachytic Do. ¹	1,291 4,559	513 2,003	759 2,201	73 18	127.583 825.591	Very small. Do.

¹ Within "doubly heterozygous" F_3 lines.



FIGURE 5.—*A*, Colseess V (normal chlorina); *B*, Brachytic (brachytic green).

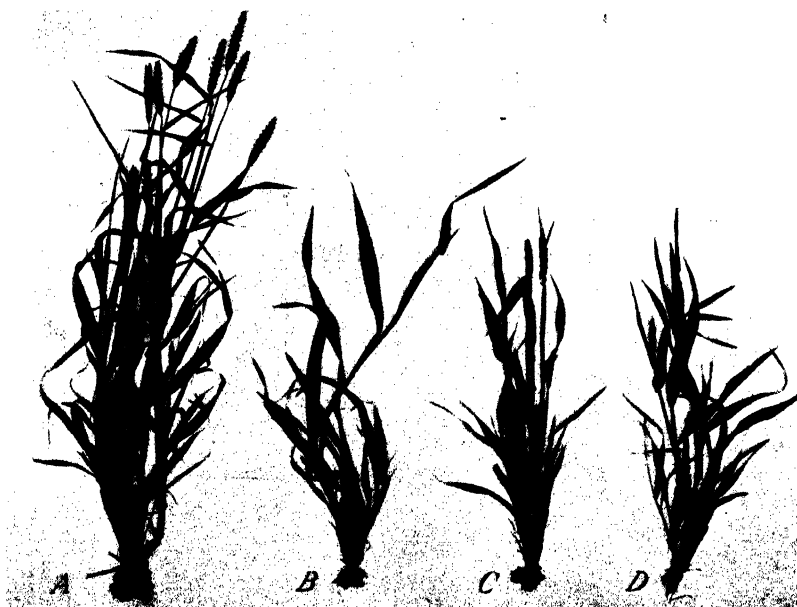


FIGURE 6.— F_3 segregates from the cross Colseess V \times Brachytic: *A*, Normal green; *B*, normal chlorina; *C*, brachytic green; *D*, brachytic chlorina.

The agreement between the observed and the expected frequencies on the basis of 30.8 percent crossing over is very poor as would be expected because of the excess of brachytic types and the deficiency of chlorina types. As has been pointed out, the drought may have been responsible for a more pronounced differential germination and emergence, the chlorina types being weaker and more susceptible to the drought. Evidence on this point is available from a comparison of the percent emergence in $F_c F_c$ with that of $f_c f_c$ F_3 lines, where the emergence percentages were 76.0 and 63.9, respectively. Since f_c is linked with Br , a deficiency of chlorina types would decrease the proportion of normal types to a greater extent than the proportion of brachytic types. The classification for Br and br phenotypes was made in the field before the plants headed; consequently, normal plants presenting a dwarfed appearance as a result of the drought might have been classified as brachytic. Mistakes of this type were occasionally encountered when the F_2 plants, saved for seed for the F_3 , were reclassified on the basis of the length of spike and awn, which is the most reliable criterion for determining the brachytic types. Other than indicating a decided linkage, the F_2 data probably do not give a very accurate cross-over value.

Of the 130 F_3 lines grown, 116 could be definitely classified according to the genotypes of the F_2 plants from which they arose. In the other 14 lines, the number of plants per line was too small to definitely establish the F_2 genotype. The minimum number of plants for accepting a line as homozygous or heterozygous was established at 12 by the χ^2 method, since only once in about 22 times would all 12 plants be of the dominant type if they arose from a heterozygous F_2 plant. Where segregation for at least three of the phenotypes occurred, the line was classified as a double heterozygote, regardless of the number of plants.

The distribution of the 116 F_3 lines is presented in table 4. Since none of the double recessives was planted, no F_3 lines appear in the last class. The separation of the $Br br F_c f_c$ types into those originating from coupling and those originating from repulsion was made by using Immer's (14) formula for determining the midpoint p' . The figures in the last column again reflect the deficiency of chlorina types which were obtained in the F_2 results.

TABLE 4.—Distribution of the F_3 lines according to the genotypes of the F_2 parental plants

F_2 genotypes	$F_c F_c$	$F_c f_c$	$f_c f_c$
$Br Br$	1	3	6
$Br br$	5	0 (coupling) 71 (repulsion)	0
$br br$	29	1	

An analysis of the F_3 data was made according to the method described by Immer (14). A summary of the analysis of the linkage relationships between br and F_c in the cross $br br F_c F_c \times Br Br f_c f_c$ as determined from F_3 data follows.

<i>F</i> ₂ genotypes	Cross-over value (per cent)
Doubly dominant.....	6.48 ± 2.03
Double heterozygotes.....	.00
Singly dominant (<i>Br f_c</i> phenotypes).....	.00
Singly dominant (<i>br F_c</i> phenotypes).....	1.69 ± 1.69
Within double heterozygotes (<i>F</i> ₂ segregation).....	9.55 ± 1.06
Combination of classes.....	9.27 ± 0.90

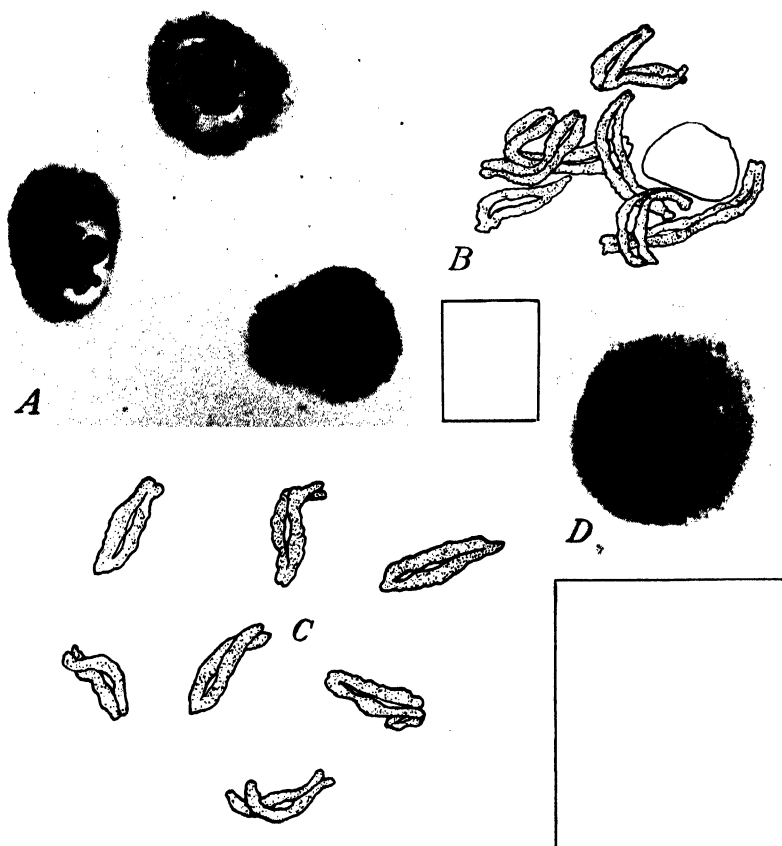


FIGURE 7.—A, Late pachynema in *F*₁ plant of Brachytic × Himalaya, × 1,125; B, chromosomes in late diplonema in *F*₁ plant of Brachytic × Himalaya, × 3,500; C, chromosomes in diakinesis in *F*₁ plant of B4 × Brachytic, × 3,500; D, microsporocyte showing bivalent (or ring?) off the equatorial plate in *F*₁ plant of Brachytic × Himalaya, × 1,695.

Because of the absence of *F*₃ lines from the *F*₂ coupling phase among the "double heterozygotes" and of *Br br f_c f_c* genotypes among the "singly dominants," zero cross-over values were obtained for the second and third classes. The fifth class may be considered as an *F*₂ segregation since all of the lines originated from *F*₂ plants in the repulsion phase. In combining all of the classes, the *F*₂ data were omitted because of the large discrepancies noted previously. The final cross-over value of 9.27 ± 0.90 percent, obtained by combining all of the

F₃ data, is the best estimate available for the linkage intensity from the F₃ repulsion data used in this study.

COMPARISON OF CHROMOSOME BEHAVIOR AND NUMBER IN BRACHYTIC AND NORMAL STRAINS OF BARLEY

A study of the early and midprophase stages in barley is rendered extremely difficult by the large amount of chromatin material contained in the nucleus and by the very small sporocytes. The chromonemata are entangled in a dense mass in late pachynema and early diplonema (fig. 7, *A*); consequently, with the technique employed, it was impossible to separate the individual pairs earlier than late diplonema or early diakinesis (fig. 7, *B*, *C*). At these stages it is difficult to obtain an accurate comparison of length between paired chromosomes, although marked differences could no doubt be detected. Further, the different pairs are indistinguishable by ordinary observation in the prophase and cannot be identified separately (fig. 7, *A*, *B*, *C*). It

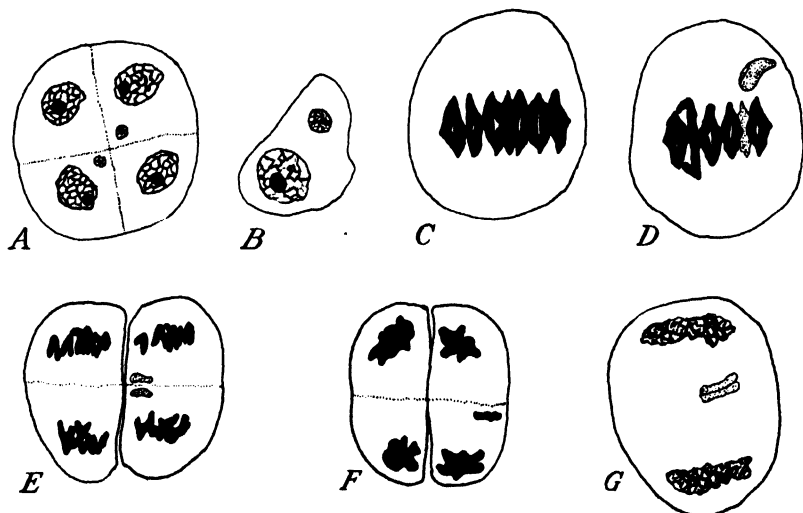


FIGURE 8.—*A*, Micronuclei in a quartet of a B4 plant; *B*, microspore with a micronucleus in F₁ plant of Brachytic \times Himalaya; *C*, normal metaphase in Brachytic; *D*, nonorientation of a bivalent in Himalaya; *E*, lagging univalent undergoing a secondary split in B4; *F* and *G*, lagging univalents in B4. In *F*, one of the halves evidently has gone to one of the poles. All \times 2,000.

therefore was necessary to make a general examination of each prophase for abnormal configurations and discrepancies in length between paired chromatids. In making these examinations, the different types of configuration noted by McClintock (24) were kept constantly in mind.

From 100 to 200 microsporocytes in the stages from pachynema to diakinesis were examined in each of the three F₁ plants of B4 \times Brachytic and in the F₁ plant of Brachytic \times Himalaya. No abnormal configurations or discrepancies in length between synapsed members of a pair were observed. However, in material of this nature, failure to observe such abnormalities does not establish the absence of observable chromosome changes but merely indicates that no gross changes have occurred (fig. 7, *B*, *C*).

From table 5 it may be seen that in 1932 the frequency of occurrence of micronuclei (fig. 8, *A*, *B*) in Brachytic was significantly lower than

in B4. The F_3 lines all were lower in micronuclei than either of the parents, although not significantly different from Brachytic. The percent frequency of micronuclei in the three F_1 plants also was lower than in the parents. There appears to be a tendency toward greater stability in the germ plasm of the hybrids than in the two varieties themselves.

The selections for high and low frequency of micronuclei from plants of B4 and Brachytic grown in 1932 appeared to be different in 1933 although the difference could not be established statistically. No effect of selection was observable in 1934 between the high and low selections from B4 plants grown in 1933.

TABLE 5.—Comparison of frequency of micronuclei in different strains and hybrids of barley, 1932–34

Year and classification	Variety or line	Plants	Micro-nuclei in mother plant	Micro-nuclei in progeny	Degrees of freedom ¹	F	Range
1932							
F ₃	{B4 × Brachytic (<i>brbr</i>)	16		0.66	4, 78	2 5.43	0.00–4.90
	{B4 × Brachytic (<i>Brbr</i>)	20		.19			.00–0.70
	{B4 × Brachytic (<i>BrBr</i>)	20		.25			.00–1.20
Parent	{B4	12		1.46			.20–5.90
F ₁	{Brachytic	15		.82			.30–2.10
	{B4 × Brachytic	3		.10			.10–0.20
1933							
Parent	{Brachytic	9	2.10	1.48	5, 45	1.03	.50–3.40
	{do	9	.30	1.38			.50–2.90
	{B4	10	5.90	2.04			.20–11.00
	{do	6	.20	.85			.40–1.50
	{Brachytic	9		.53			.00–1.90
	{Himalaya	8		.85			.00–2.40
1934							
Parent	{B4	12	11.00	1.03	1, 21	.42	.30–4.10
	{do	11	.40	1.31			.40–4.00
F ₁	{Brachytic × Himalaya	1		.40			

¹ The number of degrees of freedom for the variety or line and error mean squares, respectively.

² $P < 0.01$. 2 times the standard error of a difference between any 2 varieties or lines = 0.58 percent.

A check on a few plants having stages from metaphase I to telophase II revealed that irregularities similar to some of those occurring in wheat (27) were occasionally present. Metaphase I was nearly always of the regular type with seven bivalents uniformly distributed on the equatorial plate (fig. 8, C). In about 50 percent of the metaphase configurations, an open bivalent consisting of two equal members was observable (fig. 8, D). Whether the same pair is always involved was not definitely determined, although it appeared that the members of the open bivalent possessed a subterminal or submedian spindle attachment.

The various abnormalities shown in figure 8, E, F, G, were found to occur in all varieties and lines studied. Lagging univalents of the types shown appeared to be developing into micronuclei. Evidence of this is available from figures like those in figure 8, F, G, where chromosomes were sometimes in the process of rounding up into micronuclei after the remaining chromosomes had already been resolved into definite nuclei. The relation of other chromosome abnormalities to the occurrence of micronuclei was not determined, although in some

anthers, usually at one end or near the tapetal cells, very decided disturbances in chromosome behavior were occurring and giving rise to microspores with several micronuclei. The nonoriented bivalent or ring shown in figure 7, *D*, was found in the F_1 plant of Brachytic \times Himalaya as the only one of its kind among several hundred metaphases which were observed.

The abnormalities which have been described occur very infrequently, even in plants having high percentages of micronuclei. It would seem that in a monoploid species like barley, the loss of a chromosome would result in nonfunctional spores which would constantly be eliminated. It is therefore very doubtful whether plants lacking in a chromosome or a part of a chromosome can survive unless the part lost were very small or of an inert nature.

During the course of these studies, the chromosome numbers of Brachytic and Himalaya were checked whenever a suitable cell in metaphase I was available. The somatic numbers also were checked in the root tips used for comparing cell size and number of mitoses. In no case were deviations from the normal number of 7 pairs or 14 somatic chromosomes found (fig. 8, *C*, and fig. 9).

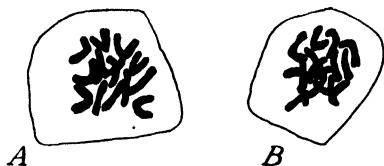


FIGURE 9.—Diploid chromosome configurations of Himalaya (*A*) and Brachytic (*B*), drawn from root tips. $\times 2,000$.

The genetic and cytologic behavior of Brachytic leads to the conclusion that the brachytic character is perhaps the result of a gene mutation, although the possibility of a minute loss or rearrangement of a part of a chromosome should not be overlooked. An extreme rearrangement such as the inversion described by Schultz and Dobzhansky (33) in *Drosophila* could no doubt have been detected in the F_1 plants of brachytic \times normal. The "position effect" resulting from a minute alteration in the chromosome as found by Muller (3) offers some possibility. For the present discussion, however, the assumption of a gene mutation will be used as a basis.

NATURE OF THE ACTION OF THE GENE FOR BRACHYTIC AS DETERMINED FROM COMPARISONS OF HIMALAYA AND BRACHYTIC

The general appearance of Himalaya and Brachytic already has been shown (fig. 1). In the comparisons which follow, an attempt has been made to compare by actual measurements some of the morphological, anatomical, and cytological characters of the two strains.

COMPARATIVE GROSS MORPHOLOGY

Typical spikes and caryopses of Himalaya and Brachytic are shown in figure 10. It will be noted that the difference in length of awns and size of caryopses is much more pronounced than the difference in length of spikes.

Comparative measurements of the two strains are presented in table 6. It is apparent that there has been a general diminution in size of the different parts of Brachytic as compared with Himalaya. Although no significant difference was obtained for weight of seed in the two samples which were compared, the writer is of the opinion that Himalaya would significantly outyield Brachytic if larger numbers were used.

The question arises as to the manner in which the brachytic gene produces the diminution in size as evidenced in the above comparisons. Possibly differences in cell size, cell number, rate of growth, or frequency of cell division might be determined as a means of explaining the differences in size of plants between the two strains.



FIGURE 10.—Comparison of spikes and caryopses of Himalaya (A) and Brachytic (B). $\times 50$.

TABLE 6.—Quantitative comparison of different parts of Himalaya and Brachytic

Gross morphological character	Himalaya	Brachytic	Difference in favor of Himalaya	Degrees of freedom	<i>t</i>	Brachytic in percent of Himalaya
Height up to tip of upper leaf ¹centimeters	65.00	42.70	22.30	8	² 8.04	65.69
Height up to base of spike ¹do	50.70	34.64	16.06	8	² 5.36	68.32
Distance between ligules ¹do	5.46	4.14	1.32	8	² 2.74	75.82
Length of leaves ¹do	22.36	14.98	7.38	8	² 11.35	66.99
Length of awns ¹do	10.91	5.70	5.21	48	² 15.86	52.29
Seeds per plant ⁴number	85.32	92.40	-7.08	48	.60	108.30
Weight of seeds ⁴grams	3.07	2.92	.15	48	.33	95.18
Weight per seed ⁴milligrams	36.76	31.36	5.40	48	² 4.20	85.31

¹ 5 plants of each variety as recorded at time of heading, June 20, 1933.

² $P < 0.01$.

³ $P < 0.05$.

⁴ 25 plants of each variety, harvested in 1934.

COMPARATIVE SIZE OF PLANTS, ORGANS, AND CELLS IN PLANTS GROWING IN THE FIELD

Two plants of each strain were studied in detail as shown in figure 11 and tables 7 and 8. Figure 11 was drawn to scale and reduced by photographing to the given size from the measurements presented in table 7 which are based on one plant of each strain, collected on June 20, 1933. The number of cells, both epidermal and parenchyma, is seen to be roughly proportional to the length of the plant and its parts, and cell length is the same in the two strains. This is further borne out in the results presented in table 8 which are based on two plants of each strain. The same plants were measured for diameter of culm, but no significant difference was found between the strains. Himalaya had a mean culm diameter of 0.39 cm. as compared to 0.37 cm. for Brachytic.

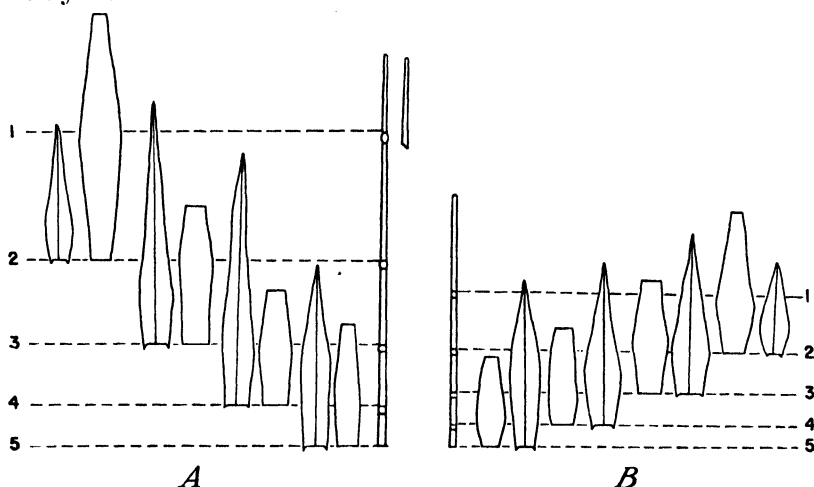


FIGURE 11.—Diagrammatic drawings to show relative lengths of different parts of Himalaya (A) and Brachytic (B). Numbers refer to specific parts studied: 1, first internode and node; 2, second internode and node, and first leaf sheath and blade; 3, third internode and node, and second leaf sheath and blade; 4, fourth internode and node, and third leaf sheath and blade; and 5, fifth internode and node, and fourth leaf sheath and blade. $\times 0.20$.

Eight pairs of comparable leaves were studied for length and width of leaves, and length and width of epidermal cells. The results presented in table 9 show that the differences in cell size between varieties were not statistically significant. It was noted in the analyses of variance which were calculated that cell length, cell width, and cell shape index (length/width) varied significantly between different regions of the leaf. These comparisons are presented in table 10. The cells are longer and narrower in the terminal region and shorter and wider in the basal region, the middle region being intermediate. Cell shape also varied significantly between leaves, the older leaves having longer and narrower cells. There evidently is some correlation between cell shape and shape of different parts of the leaf and between cell shape and age of leaf.

Apparently the brachytic gene has not affected cell size in mature plants. Although the number of plants compared is very small, the

pronounced difference in size between Himalaya and Brachytic and the use of comparable plants appear to justify the comparisons which have been made.

TABLE 7.—Comparison of length, and number and length of epidermal cells in different parts of Himalaya and Brachytic, one plant of each strain as shown in figure 11

Part of plant	Length of parts		Cells per part		Length of cells	
	Himalaya	Brachytic	Himalaya	Brachytic	Himalaya	Brachytic
	<i>Centimeters</i>	<i>Centimeters</i>	<i>Number</i>	<i>Number</i>	μ	μ
First internode.....	17.41	9.27	1,454	768	120	121
Second internode.....	11.88	5.10	995	420	119	121
Third internode.....	7.72	3.89	661	320	117	122
Fourth internode.....	5.95	2.48	470	207	127	120
Fifth internode.....	3.31	1.70	271	145	122	117
Mean.....	9.25	4.49	770	372	121	120
First node.....	.48	.45	58	48	83	94
Second node.....	.49	.45	60	51	82	88
Third node.....	.49	.49	60	61	82	80
Fourth node.....	.45	.40	53	48	85	83
Mean.....	.48	.45	58	52	83	86
First leaf sheath.....	23.95	14.61	1,844	1,135	130	129
Second leaf sheath.....	13.50	10.99	1,007	833	134	132
Third leaf sheath.....	11.22	9.35	860	728	130	128
Fourth leaf sheath.....	10.70	8.76	841	676	127	130
Mean.....	14.84	10.95	1,138	843	130	130
First leaf blade.....	13.00	9.00	843	596	154	151
Second leaf blade.....	23.50	15.30	1,465	911	160	167
Third leaf blade.....	24.40	16.90	1,510	971	162	174
Fourth leaf blade.....	21.50	16.50	1,337	969	161	170
Mean.....	20.60	14.43	1,289	862	159	166

TABLE 8.—Comparison of mean length, and mean number and length of cells in analogous parts of Himalaya and Brachytic, two plants of each strain

Part of plant	Mean length of parts		Mean number of cells per part		Mean length of cells	
	Himalaya	Brachytic	Himalaya	Brachytic	Himalaya	Brachytic
	<i>Centimeters</i>	<i>Centimeters</i>	<i>Number</i>	<i>Number</i>	μ	μ
Culm (epidermal cells).....	31.39	16.32	2,661	1,418	118	115
Internodes.....	7.48	3.73	620	311	120	120
Nodes.....	.42	.40	52	49	80	81
Culm (parenchyma cells).....	31.39	16.32	1,810	1,002	173	163
Internodes.....	7.48	3.73	410	210	182	178
Nodes.....	.42	.40	49	46	86	87
Leaf sheaths (epidermal cells).....	14.66	11.23	1,183	762	130	136
Leaves (epidermal cells).....	21.55	14.99	1,749	880	159	169

TABLE 9.—Comparison of mean length and width of leaves, and length and width of epidermal cells in Himalaya and Brachytic, eight leaves of each

Character	Himalaya	Brachytic	Difference ¹	t
Length of leaves..... centimeters	12.16	9.80	2.36	² 7.00
Width of leaves..... do.....	.56	.56		
Length of cells..... μ	173.13	184.83	-11.70	1.36
Width of cells..... μ	30.50	30.38	.12	.14
Cell-shape index, $\frac{\text{length}}{\text{width}}$	5.75	6.13	-.38	.99

¹ 7 degrees of freedom in each instance.

² $P < 0.01$.

TABLE 10.—Comparison of width of leaf, and width, length, and shape of epidermal cells in different regions of the leaf in Himalaya and Brachytic

Character	Region of leaf			F
	Base	Middle	Terminal	
Width of leaf centimeter	0.61	0.84	0.23	¹ 79.25
Width of cells μ	31.50	30.70	29.50	¹ 10.89
Length of cells μ	169.90	175.80	193.30	² 5.23
Cell-shape index, $\frac{\text{length}}{\text{width}}$	5.38	5.70	6.78	¹ 15.33

¹ $P < 0.01$.² $P < 0.05$.

COMPARATIVE SIZE OF ORGANS AND CELLS IN THE PLUMULES AND PRIMARY ROOTS

The mean lengths of the plumules and their constituent cells are presented for 12 plants of each variety in table 11. It will be noted that cell length in the primary leaf is significantly different between the two varieties. On further examination of the analysis of variance, it was found that the entire difference could be accounted for by the middle region as shown in table 12. The segment from the middle region had been taken at the midpoint between the base and tip of the primary leaf; consequently, the middle segment of Himalaya was farther from the base than the middle segment of Brachytic and might be expected to have longer cells. Had the segments been taken at equal distances from the base in the two varieties, it is doubtful whether a difference in the cell size would have been found. Apparently the cells approach a maximum length in the terminal region, for here the two varieties are again similar. The fact that older leaves have much longer cells indicates that the maximum attained in young leaves is only of a temporary nature.

As in the plumules, the difference between Himalaya and Brachytic in length of primary roots approaches 50 percent. The comparisons in table 13 indicate that the width of the primary roots is significantly greater in Brachytic and that the difference in width is largely the result of a greater number of cells in this dimension, although there is also some indication of wider cells. Length of cells and cell shape were similar in the two varieties, although they were greatly different in the two regions.

TABLE 11.—Comparison of length of plumules, and length of cells in coleoptiles and primary leaves of Himalaya and Brachytic, 12 plants of each variety

Character	Himalaya	Brachytic	Difference	Degrees of freedom ¹	F
Length of plumule millimeters	56.25	32.42	23.83	1, 16	² 37.54
Length of cell in coleoptile μ	174.08	186.63	-12.55	1, 16	.82
Length of cell in primary leaf μ	34.21	29.44	4.77	1, 16	³ 5.46

¹ 1, 16 the number of degrees of freedom for the varietal and error mean squares, respectively.² $P < 0.01$.³ $P < 0.05$.

TABLE 12.—*Comparison of length of cells in microns in different regions of the primary leaves of Himalaya and Brachytic*

Leaf region	Himalaya	Brachytic	Difference	Degrees of freedom ¹	F
Basal.....	18.50	17.61	0.89	2, 61	23.61
Middle.....	44.04	32.56	11.48		
Terminal.....	40.10	38.15	1.95		

¹ The number of degrees of freedom for variety × region and error mean squares, respectively.² $P < 0.01$, 2 times the standard error of a difference between varieties in any region = 6.14μ .TABLE 13.—*Comparison of length and width, and cell size and shape in primary roots of Himalaya and Brachytic*

Character	Himalaya	Brachytic	Difference ¹	F
Length of primary root..... millimeters	21.50	10.30	11.20	232.89
Width of middle region..... do	.32	.42	— .10	17.99
Cells across middle region..... number	10.30	12.80	— 2.50	8.81
Width of cells in middle region..... μ	30.80	33.09	— 2.29	.65
Length of cells in middle region..... μ	129.13	123.16	5.97	.10
Cell-shape index in middle region..... μ	4.33	3.91	.42	.55
Width of tip..... millimeters	.32	.30	— .02	226.24
Cells across tip..... number	20.30	22.30	— 2.00	2.13
Width of cells in tip..... μ	15.90	17.60	— 1.70	2.57
Length of cells in tip..... μ	26.98	20.22	— 6.76	1.56
Cell-shape index in tip..... μ	1.70	1.60	.10	

¹ In all cases the number of degrees of freedom for the varietal and error mean squares are, respectively, 1 and 12.² $P < 0.01$.³ $P < 0.05$.

From the comparison of the gradients in number of cells per field in table 14, it appears that the cells approach a constant for cell length at about the same level in the two varieties. There is, however, a tendency for Himalaya to have a larger number of cells in fields 3 and 4. This is the line between the region of cell division and the region of cell elongation. If Himalaya has a higher rate of cell division, as is shown later, it might be expected to show an accumulation of cells in this region.

The evidence from the plumules and primary roots substantiates the conclusion from plants growing in the field that cell size in Brachytic is similar to cell size in Himalaya. It would seem that the difference in plant size between Himalaya and Brachytic might be fully accounted for by a difference in cell number.

COMPARATIVE DAILY GROWTH INCREMENTS

The growth measurements taken during the period from May 20 to June 20 represented 11 successive intervals, varying in length from 2 to 6 days. The means for the five plants of each variety are presented for each interval with the total and mean daily growth increments for the entire period in table 15. As expected, the difference between varieties is consistently in favor of Himalaya except in the first interval where, apparently by chance, Brachytic has grown more than Himalaya.

Although there is a striking difference in the amount of growth from day to day, the time of differentiation of the various parts and the date of heading remain practically the same in the two varieties. This is shown in table 16, which is based on observations of comparable

rows grown in 1933. Apparently the brachytic effect is manifested only in the daily amount of growth and the total amount of growth attained. Other factors tend to produce their effects independently, regardless of the height or size of the plant.

TABLE 14.—Comparison of cell-number gradients in root tips of Himalaya and Brachytic

Field No.	Cells per field in—		Differ- ence	Field No.	Cells per field in—		Differ- ence
	Himalaya	Brachytic			Himalaya	Brachytic	
	Number	Number	Number		Number	Number	Number
1.....	27.3	26.9	0.4	7.....	2.4	2.7	-0.3
2.....	29.8	29.0	.8	8.....	2.1	2.8	-.7
3.....	21.0	14.9	6.1	9.....	1.8	2.5	-.7
4.....	9.6	6.0	3.6	10.....	1.8	2.2	-.4
5.....	4.7	3.7	1.0	11.....	1.7	2.1	-.4
6.....	3.1	3.1	.0	12.....	1.6	1.6	.0

TABLE 15.—Comparison of mean daily growth increments of Himalaya and Brachytic

Variety	Mean daily growth during intervals shown										Total growth	Mean daily growth
	May 20-22	May 23-24	May 25-30	May 31- June 1	June 2-3	June 4-5	June 6-7	June 8-10	June 11-14	June 15-17	June 18-20	May 20- June 20
Himalaya.....	Cm. 0.70	Cm. 2.07	Cm. 1.40	Cm. 1.53	Cm. 2.05	Cm. 2.10	Cm. 1.98	Cm. 1.85	Cm. 1.44	Cm. 2.83	Cm. 2.37	Cm. 50.18
Brachytic.....	1.23	.85	1.17	1.20	.60	1.50	1.47	1.03	1.00	1.44	1.45	30.58
Difference.....	-.53	1.22	.23	.33	1.45	.54	.51	.82	.44	1.39	.92	19.60

¹ The mean growth increment of Brachytic is 65.19 percent that of Himalaya.

TABLE 16.—Comparison of dates of differentiation and date of heading of Himalaya and Brachytic

Variety	Planting	Emer- gence	Appear- ance of second leaf	Appear- ance of third leaf	Appear- ance of fourth leaf	Appear- ance of fifth leaf	Appear- ance of first tiller	Head- ing
Himalaya.....	Apr. 28	May 10	May 13	May 17	May 21	May 24	May 22	June 20
Brachytic.....	Apr. 28	May 11	May 13	May 17	May 22	May 24	May 24	June 20

COMPARATIVE FREQUENCY OF MITOSIS

The meristematic region in the root tips of Himalaya was found to be significantly longer and narrower than that of Brachytic. The two dimensions were compensatory as far as the two varieties were concerned, for the total area per section was similar. Since cell size was not significantly different between varieties, the total number of mitoses per section was compared directly. The number of mitoses per square millimeter also was figured for each plant in order to obtain a comparison on a unit-area basis. The results are presented in table 17.

The frequency of mitosis is somewhat higher in Himalaya for both total number of mitoses per section and number per square millimeter,

indicating that the rate of cell division might be higher in Himalaya than in Brachytic. Owing to the large amount of variation within varieties, however, the difference between varieties could not be established as being statistically significant. The use of a larger number of plants would be necessary to determine this more accurately. Since cell size in the meristematic region is similar in the two varieties, it seems reasonable to assume that the critical size for cell division also is similar. Consequently, cell elongation and cell enlargement might be expected to occur at a higher rate in Himalaya to bring the cells to the critical size for division more rapidly.

TABLE 17.—*Comparison of area per section of meristematic region in root tips, and frequency of mitosis per section in Himalaya and Brachytic*

Character	Himalaya	Brachytic	Difference ¹	F
Length..... millimeters..	0.671	0.540	0.131	2 15.81
Width..... do.....	.035	.042	-.007	2 13.41
Area..... square millimeters..	.215	.207	.008	.63
Mitoses per section..... number..	65.2	51.4	13.8	1.69
Mitoses per square millimeter..... do.....	303.4	245.8	57.6	2.30

¹ In all cases the number of degrees of freedom for the varietal and error mean squares are, respectively, 1 and 12.

² $P < 0.01$.

DISCUSSION

The small number of chromosomes in cultivated barley makes it ideal material for studies of inheritance and linkage. The fact that it is a simple diploid is good reason for believing that a large proportion of its characters will be inherited in a simple Mendelian manner. The work on barley cited in the review of literature shows rather conclusively that such is the case. Stadler (40, 41) concluded from his results in inducing mutations by X-rays in diploid, tetraploid, and hexaploid species of small grains, that the lower mutation rates in polyploid species are due to the presence of duplicate or triplicate factors, all of which must mutate for at least one member of the factor pair in order that the mutation be expressed in the resulting progeny. Cultivated barley was shown to have a significantly higher mutation rate than the cultivated species of wheat and oats. It follows, therefore, that very little duplication of genes in different chromosomes is to be expected.

It is due to the work of Robertson, Deming, and Koonce (31) that the seventh linkage group in barley has been established. These workers have to date located two genes in this group, both of them chlorophyll abnormalities which are either lethal or lacking in survival ability when in the homozygous recessive condition. The location of genes for characters having normal survival ability in this linkage group therefore would obviate difficulties encountered when testing for the presence of other genes. The gene for brachytic should be of value in this respect since Brachytic plants have good survival ability.

From a cytogenetic standpoint, it would seem that difficulties will be encountered in attempts to correlate genetic and cytologic data for the different linkage groups in barley as has been done in *Zea* (21, 24). The chromosome strands in barley are very densely interwoven in the midprophase stages and the chromosome pairs are very similar in appearance and size. It therefore is difficult to separate

and distinguish the different pairs, especially in the stages desired. Lewitsky (19), however, has reported that the somatic chromosomes in barley may be differentiated on the basis of precise measurements and the presence of constrictions in certain regions of the chromosome after special fixing and staining techniques are employed. Special techniques for stages of meiosis or a study of the somatic chromosomes therefore offer possibilities for cytogenetic studies in barley.

The manner in which a gene expresses itself as manifested in its effect on the mature organism is of considerable importance in determining the action or dynamics of the gene. For example, what are the intermediate steps between the immediate processes of the gene after it enters the zygote and the ultimate effects which it produces on the mature individual?

The relation of genetic factors to enzyme production has been discussed by Goldschmidt (8, 10). Van Overbeek (42) showed that a simply inherited dwarf type of growth in corn was the result of a higher rate of destruction of growth substance (growth hormone or auxin) in the dwarf type than in the normal. It is conceivable that the difference in the amounts of growth attained in Himalaya and Brachytic may be due to a heritable difference in the amount of growth substance produced or destroyed.

Goldschmidt (9) has postulated genetic factors which determine the time and rate at which developmental processes occur. A comparison between Himalaya and Brachytic is of interest in this respect. Growth occurs at different rates, but differentiation and maturation occur at the same time in both varieties. The gene for the rate of growth, which may be considered a rate factor, evidently produces its effects independently of genes for differentiation which may be considered time factors. Further evidence on the independence of genes for growth and differentiation is available from the 24 F_3 lines of $B4 \times$ Brachytic. $B4$ was about 5 days earlier for date of heading than Brachytic in 1932; however, one homozygous brachytic F_3 line was obtained which headed 1 day earlier than any of the $B4$ lines and 6 days earlier than any of the Brachytic lines.

The results indicate that the organism as a whole rather than the individual cell must be considered as the fundamental unit of development. Factors for growth and differentiation seemingly act on the entire plant structure which during the course of its development becomes differentiated into cells whose number and form are determined by the size and form which the plant and its organs attain. The extent to which cells are formed seems to have no bearing on the time of differentiation and maturity; factors determining these processes evidently act on the entire protoplasmic mass, regardless of the number of cells which have been formed. This conclusion may have some bearing on the controversy between the "cell theory" and "organismal theory" which has been reviewed and discussed by Sharp (34) and Sinnott (36).

SUMMARY

A mutant character in barley, designated as brachytic, has been studied with respect to its genetic and cytologic behavior.

The gene for brachytic habit of growth behaves as a simple Mendelian recessive to the gene for normal habit of growth. It has been designated as *br*.

The factor pair *Br br* was found to be independent of the first six linkage groups and definitely associated with *F_c f_c* in the seventh linkage group.

The linkage intensity between *Br br* and *F_c f_c* was determined from repulsion data. The cross-over value from the *F₂* data was found to be 30.8 ± 1.8 percent but is not considered reliable because of a deficiency of chlorina types and possible errors in classification. The cross-over value from the *F₃* data, which may be considered more reliable, was 9.27 ± 0.90 percent.

The appearance, behavior, and number of chromosomes were as regular in Brachytic as in the normal strains, Himalaya and B4. The brachytic character is not associated with any observable change in chromosome structure; more likely it is the result of a gene mutation.

There is a general diminution in size of Brachytic plants and their parts as compared to those of Himalaya, the variety from which Brachytic mutated. Brachytic grows about two-thirds as tall, its leaf sheaths are about two-thirds as long, and its awns are about one-half as long. The number of seeds per plant is about the same for the two strains but Brachytic seeds are only 85 percent as heavy. The total weight of seed per plant in Brachytic was about 95 percent of that for Himalaya but the standard error of the difference was too large to establish a significant difference.

Length of cells in comparable plants growing in the field was found to be similar in the two strains. The number of cells is proportional to length of the plant and its parts.

Epidermal cells were longer and narrower in the tip than in the middle and basal regions of the leaves. Older leaves had longer and narrower cells than younger leaves.

Although the plumules and primary roots of Himalaya were approximately twice as long as those of Brachytic, no significant differences were obtained for cell size in comparable parts.

The primary roots of Brachytic were larger in diameter than those of Himalaya, the difference being associated more strongly with number than with width of cells.

Cells in the primary leaves and primary roots increased progressively in size at increasing distances from the meristematic region. The nature of the gradient in root tips differed somewhat between strains in the region differentiating the zone of cell division and the zone of cell elongation, but cell size was found to be similar in the two strains beginning only a short distance back of this region.

Brachytic grew 65.19 percent as tall as Himalaya, the difference in daily amounts of growth remaining fairly constant between the two varieties.

Factors for differentiation and maturity appeared to act independently of the factor for rate of growth.

Himalaya had a longer and narrower meristematic region in sections of root tips than Brachytic but the total area per section was similar in both varieties.

The results indicate that Himalaya has a higher frequency of mitosis per section of meristematic region than Brachytic but this could not be established statistically. A similar relationship was found for the number of mitoses per square millimeter. The rate of cell division and elongation might be expected to be higher in Himalaya than in Brachytic.

A discussion of linkage groups and the possibility of correlating genetic and cytologic data in barley has been presented.

The relation of genetic factors to the production and utilization of growth substances, which in turn influence the rate of growth and cell elongation, has been discussed.

An explanation based on Goldschmidt's physiological theory of inheritance has been advanced to account for the independence of the gene for rate of growth and factors for differentiation and maturity.

The results indicate that cell formation is subordinate to the development of the organism as a whole.

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JOURNAL OF AGRICULTURAL RESEARCH

VOL. 60

WASHINGTON, D. C., JUNE 1, 1940

No. 11

HYBRID VIGOR IN SUGAR BEETS

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INTRODUCTION

Hybrid vigor in first-generation crosses is a fundamental consideration in present-day methods of plant improvement. If it can be established that marked vigor rather generally occurs when inbred strains of sugar beet (*Beta vulgaris* L.) are crossed and that hybrids can be obtained which are superior to open-pollinated varieties, such findings would have important directive influence upon methods of sugar-beet breeding.

This report gives the results of field tests in which the sugar-beet hybrids arising as single crosses of inbred strains or as top crosses of inbred strains on an open-pollinated variety are compared with the parental sorts.

MATERIAL AND METHODS

The production by selection and inbreeding of the strains used in this study was begun by the late W. W. Tracy, Jr., in 1915 and continued by him until 1929, when the present writers took over the project. As a rule, a selfed generation was obtained every 2 years by bringing individual plants to seed either in home gardens some distance from each other or on widely separated farm locations. As the work proceeded, it was a general practice to test a number of sister progenies of a strain in each generation, discarding those considered undesirable. From the large collection of strains arising in Tracy's work, those listed in table 1 were chosen for crossings and tests for hybrid vigor. The original source or accession number of 1915 for a strain is given with the salient points of the breeding history. For easy reference in the text, the strains have been assigned Arabic numbers. Crosses between strains are designated in the customary manner, the seed-bearing parent being shown first.

To produce a hybrid, the two strains to be crossed were brought to seed production in a location at considerable distance from any other seed beets. The propensities of the unrelated strains to cross-pollinate reciprocally were relied upon to effect hybridization. In most cases the seed harvest was according to the strain serving as seed bearer, thus giving reciprocal crosses from each location. The sugar beet is known to be generally cross-pollinated under natural conditions, although selfing can be induced with a rather high percentage of plants taken from heterozygous populations. Completely self-sterile segregates or individuals that for other reasons fail to set seed drop out of the breeding material as inbreeding continues; hence, only lines with some degree of self-fertility were available in the breeding stock. In the progenies grown from the various hybrid

¹ Received for publication January 24, 1940.

² Resigned November 30, 1933.

seed lots there occurred not only F_1 plants but selfs of the strain that was seed bearer. Where distinctive characters were established in the parents, knowledge of the mode of inheritance was useful in identifying hybrid plants in the populations. In such cases the data are based upon the performance of the F_1 class of plants. With many of the progenies it has been necessary to include a considerable proportion of selfs; in others, the entire population, regardless of the actual extent of hybridization, has been taken. For this reason it is thought that in the considerations of performance in these cultures the comparisons with the strain serving as seed bearer are the most critical as well as the most conservative.

TABLE 1.—Strains used in the production of hybrids, with salient points of their breeding history

Designation of strain in text ¹	Generations		Seed lot used	Accession No. or source ²	Designation of strain in text ¹	Generations		Seed lot used	Accession No. or source ²
	Selfed	Grouped				Selfed	Grouped		
	Number	Number				Number	Number		
1	5	2	00560-0	1948.	9b	6	2	00203-0	1846.
2	6	2	4793-29	1846.	9c	7	1	10431-0	1846.
3	5	2	3444-29	1991.	10	5	1	100888-27	1899.
4a	5	2	506-28	2140.	11	3	3	3786-29	1736-6.
4b	4	3	4874-29	2140.	12	7	1	1223-29	280-32.
5a	6	2	6484-29	2073.	13	6	1	4563-27	2125.
5b	5	2	13844-27	2073.	14a	4	3	4842-29	1700-1.
6	7	1	1294-29	1892.	14b	5	2	6326-29	1700-1.
7	6	2	6571-29	2092.	15	1	0	115768-27	LSR. ³
8a	5	3	2889-29	1910.	16	1	1	00383-0	La Royale. ⁴
8b	5	3	3063-29	1910.	17			Pioneer	Commercial. ⁵
9a	6	1	10742-27	1846.					

¹ Subgroups, representing different selections from essentially the same genetic complex, have been designated as a, b, or c.

² Strains 1 to 14, inclusive, trace to mass-selected breeding stocks received by W. W. Tracy, Jr., in 1915 from F. J. Pritchard, who had accumulated the material during his sugar-beet investigations. There was no history of inbreeding prior to Tracy's work.

³ Leaf Spot Resistant, a variety developed by the agricultural research department of the American Crystal Sugar Co. under the supervision of Skuderna (6).^{*}

⁴ A commercial brand of K. Buszczynski & Sons, Ltd., Warszawa (Warsaw), Poland.

⁵ A commercial brand of Zuckerfabrik Kleinwanzleben, Kleinwanzleben, Germany.

^{*} Italic numbers in parentheses refer to Literature Cited, p. 738.

In obtaining the field data on performance, the roots were washed free of adhering soil, permitted to dry, and then weighed and counted to determine the average root weight. The roots were then individually split, one-half of each root being used in a composite plot sample for determining sucrose percentage in the pulp by the Sachs-Le Docte cold-water-digestion method. From the average root weight of a plot and the sucrose percentage of the plot sample, the average gross sugar per root was calculated for each plot. From the appropriate plot values, the mean for each strain or hybrid was obtained. For convenience in making comparisons of performance, the data for root weight and gross sugar have been calculated, by use of a factor for stand,³ to show acre yields.

FIELD TESTS

For the field tests at Fort Collins, Colo., seed obtained from the various crosses and the parental strains was grouped according to

³ 29,094, based upon stand counts of Pioneer for all tests.

relationship. There were 5 groups made up as shown in table 2 as tests 1, 2, 3, 4, and 5. The variety Pioneer,⁴ commonly classed as a sugar type, was included in each test as a representative of non-inbred stock. The seed lot smallest in quantity determined the extent to which all within a given test could be planted. In test 1, all plots were 3 rows wide. It was necessary to restrict the plot width to a single row for 4 of the 6 replications in tests 3 and 4, and in 6 of the 8 replications in tests 2 and 5. Otherwise, three-row plots were used in these tests. Where single-row plots occurred, every alternate row was planted with Pioneer to give common competition. The experimental plots (26 feet long) were arranged for each of the 5 tests according to the equalized randomized-block scheme suggested by Student (8)⁵ as a modification of the Latin square. The analysis of variance as given by Fisher (4) for a Latin square was used in statistical reduction of data. Shortly before harvest, the plants at the ends of each plot and those that bordered gaps along a row to be sampled were cut out. The remaining plants were harvested, only the center row in the three-row plots being used. In the case of a complete stand, there were 25 plants per plot; however, taking into account elimination of selfs and very occasional skips, the plots generally provided only 10 to 20 roots. The data for yield of roots, sucrose percentage, and yield of gross sugar as obtained in the tests are given in table 2.

For each of the economic attributes, insofar as comparisons can be made, the hybrid or the hybrid population plus selfs is compared with (1) mother strain, (2) pollen strain, (3) mean of both parents, and (4) the commercial brand, Pioneer. These comparisons are given as percentage values, where 100 is equality and values above or below represent performance either superior or inferior to the base chosen. The percentage values in terms of the performance of the commercial brand may be used as coefficients of performance for comparisons of strains or hybrids in one test with those occurring in another, since the commercial brand was included in each test.

CERCOSPORA LEAF SPOT

Late in July an epidemic of cercospora leaf spot (*Cercospora beticola* Sacc.) occurred and continued until harvest. This disease is known to influence the growth of the sugar beet, depressing root yield and sucrose percentage. Since the parental strains differed in resistance, a few being very susceptible, a factor of considerable importance was introduced. For certain crosses the response attributed to hybrid vigor would undoubtedly be less pronounced were tests conducted under disease-free conditions. Specific examples of crosses of this type are discussed later.

⁴ One of the several commercial brands of foreign sugar-beet seed sold in this country at the time these investigations were initiated.

⁵ Italic numbers in parentheses refer to Literature Cited, p. 738.

TABLE 2.—Comparisons, in performance, of hybrids with their respective parental strains and with the commercial brand Pioneer¹ in tests at Fort Collins, Colo., 1932

TEST 1. RESULTS BASED ON EIGHT-PLOT AVERAGES

Parent or hybrid	Yield of roots			Sucrose			Gross sugar		
	Value expressed as percentage of—			Value expressed as percentage of—			Value expressed as percentage of—		
	Average weight of root	Fe-male	Fe-male + Pioneer	Average in roots	Fe-male	Fe-male + Pioneer	Average yield per root	Fe-male	Fe-male + Pioneer
Parents:	Pounds			Percent			Pounds		
Strain 9a	0.9874			14.05			0.13433		
Pioneer (17)	.9311			11.10			.10333		
Hybrids:	.9305			13.00			.12092		
1 (row 1) × 9a	1.6393	173	178	13.08	93	104	21697	6.313	162
1 (row 3) × 9a	1.4900	156	158	13.34	95	120	19789	5.757	147
1 (row 5) × 9a	1.5029	21.86	159	162	13.14	104	19712	5.735	147
1 (row 7) × 9a	1.3538	19.69	141	145	14.13	101	19106	5.559	142
1 (row 9) × 9a	1.5880	23.10	166	171	13.41	95	21307	6.199	159
1 (row 11) × 9a	1.5688	22.82	164	168	13.06	93	20461	5.953	152
Average	1.5271	22.22	160	164	13.36	95	20346	5.919	151
9a ₁ × 1	1.3403	144	142	144	112	98	16541	4.812	123
9a ₂ × 1	1.6126	23.46	173	168	12.49	113	20219	5.883	196
9a ₃ × 1	1.5890	23.12	171	166	12.51	113	19904	5.791	193
9a ₄ × 1	1.7129	24.92	184	179	12.45	99	21325	6.204	205
9a ₅ × 1	1.5124	22.00	162	158	12.80	115	19192	5.584	136
Average	1.5534	22.60	167	162	12.53	103	19436	5.655	138
1 × Pioneer (17)	1.1714	122	126	126	104	112	17037	4.957	127
Pioneer (17) × 1	1.0423	15.16	112	109	110	102	14399	4.189	119
Average	1.1068	16.10	117	119	105	109	15718	4.573	123
Difference required for significance ¹	.2100	3.05		.45			.02784	.810	

TEST 2. RESULTS BASED ON EIGHT-PLOT AVERAGES

[illegible]

TEST 3. RESULTS GIVEN AS SIX-PLOT AVERAGES

Parents:									
0.7280	10.59							0.10830	3.150
Strain 1	14.82							4.498	3.355
Strain 2	1.0188							12.719	3.760
Strain 4b	8.627							3.418	3.418
Strain 7	8.627							1.729	3.812
Strain 8a	8.627							1.729	3.812
Strain 15	9.640							1.150	3.24
Pioneer (17)	8.805								
Hybrids:									
1×2 ^a	16.96	134	142	14.45	97	106	16810	4.801	151
1.3637	18.96	179	149	15.50	98	97	18835	5.180	169
1.0663	15.22	103	141	14.43	96	98	14551	4.232	145
Reciprocal	1.0463	120	128	14.00	95	93	14551	4.232	112
$1 \times 8a$	1.2127	167	143	15.4	98	102	17899	5.208	133
1×15	1.0772	15.67	148	14.73	98	102	15070	4.384	161
$4b \times 1$	1.9072	14.51	112	13.98	103	97	14596	4.247	133
$4b \times 15$	1.2753	18.55	143	14.67	103	98	14596	4.247	133
7×1	1.1888	13.7	123	13.75	96	100	17527	5.069	132
$7 \times 8a$	1.2015	19.39	163	15.5	93	92	16871	4.763	157
Reciprocal	1.2038	17.48	141	14.46	93	91	16871	4.763	147
$8a \times 7$	1.1413	16.60	152	15.8	87	83	14336	4.171	129
		135	134	13.9	86	82	15297	4.451	124
				12.85	93	89	14654	4.263	119
Difference required for significance ^a									
1.528	2.22			52			0.2184	635	

See footnotes at end of table.

TABLE 2.—Comparisons, in performance, of hybrids with their respective parental strains and with the commercial brand Pioneer¹ in tests at Fort Collins, Colo., 1932.—Continued

TEST 4. RESULTS GIVEN AS SIX-PLOT AVERAGES

Parent or hybrid	Yield of roots			Sucrose			Gross sugar		
	Aver- age weight of root	Value expressed as per- centage of—		Value expressed as per- centage of—		Value expressed as per- centage of—			
		Fe- male	Male	Fe- male $\frac{\text{male}}{2}$	Fe- male	Male $\frac{\text{male}}{2}$	Fe- male terminal strain	Acre yield (calcu- lated) ²	Mean of both par- ents
Pounds	Tons	Percent	Pounds	Pounds					
Parents:									
Strain 1	0.7582	11.03							
Strain 5a	0.9797	14.25							
Strain 8b	.9688	14.38							
Strain 10	.9498	13.82							
Strain 14a	.7618	11.08							
Strain 14b	.7772	11.31							
Pioneer (17)	.8355	12.15							
Hybrids:									
1 × 5a	1.3822	20.11	182	141	159	165	98	105	
5a × 10	1.8882	23.12	210	162	183	190	91	92	
Reciprocal	1.0645	12.92	92	94	92	101	109	105	
8b × 5a	1.0645	15.49	112	109	110	103	95	99	
8b × 1	.9173	13.34	93	94	93	110	14.06	99	
8b × 10	1.0204	18.94	132	172	149	14.13	100	94	
(8b × 10) ³	1.0204	18.94	132	172	149	14.13	100	94	
8b × 14b	1.0173	14.86	103	131	105	122	13.07	101	
10 × 14b	1.0555	13.90	101	123	111	122	14.42	94	
Reciprocal	1.0620	14.58	129	105	116	120	12.82	99	
14a × 1	.9215	13.41	121	122	121	110	13.53	97	
Difference required for significance ⁴	.1920	2.79					.58		

TEST 5. RESULTS GIVEN AS EIGHT-PLOT AVERAGES

Parents:	0.7843	11.41				14.25			0.11146	3.243	
Strain 1	0.8180	11.90				15.15			.12380	3.605	
Strain 3	1.0491	15.26				12.30			.12792	3.722	
Strain 4a	1.0655	15.50				13.08			.13836	4.055	
Strain 5b	1.2258	17.83				13.39			.16318	4.748	
Strain 6	1.7228	10.51				12.58			.09065	2.637	
Strain 9c	1.1670	16.98				13.16			.15342	4.464	
Strain 12	.9975	14.51				11.61			.11560	3.363	
Strain 13	.9441	13.73				13.46			.12720	3.701	
Pioneer (17)											
Hybrids: ¹											
(1 × 3)	1.1203	16.30	140	119	14.28		97	106	.15730	4.576	134
(1 × 6)	1.3939	20.28	139	148	13.21		99	98	.18401	5.354	145
(1 × 6)	1.3055	18.99	130	138	13.34		97	99	.17465	5.081	137
(1 × 12)	1.3241	19.26	136	140	13.44		98	100	.17829	5.187	140
(4a × 5b)	1.1073	16.11	105	117	12.96		102	96	.14318	4.166	113
(4a × 12)	1.2975	18.87	117	137	12.35		97	92	.15989	4.652	114
(5b × 9c)	1.3736	19.98	134	145	12.63		95	94	.17382	5.060	137
(6 × 9c)	1.3990	20.35	144	148	12.00		92	89	.16677	4.852	131
(6 × 13)	1.2093	17.59	109	128	11.35		91	84	.13502	3.928	97
Difference required for significance ¹	.1778	2.59			.56				.02197	.639	

¹ Produced by the Zuckerfabrik Kleinwanzleben, Kleinwanzleben, Germany.² Computed from average individual root values to acre basis by assuming a stand per acre of 29,084 plants.³ This value for stand was obtained by averaging the stand of all check plots in the field.⁴ Obtained by averaging individual plot values, hence differing slightly from products of means for average root weight and sucrose percentage shown.⁵ Progenies of sibs of strain 9a were the respective seed bearers in this series of crossings and are designated by the subscript numerals 1, 2, 3, etc. Seed from strain 1 was kept separate by rows.⁶ Twice the standard error of the difference of 2 means.⁷ Seed from strains entering the cross pooled.

RECIPROCAL CROSSES OF STRAINS 1 AND 9a

The single cross between strains 1 and 9a was considered very desirable for a detailed study of the vigor of hybridity in the sugar beet. In previous tests each inbred had shown striking phenotypic uniformity (fig. 1). Numerous progenies of each had been grown and studied under field conditions. These always adhered closely to the parental strain type, predicated an approach to homozygous condition



FIGURE 1.—Four-row plots of strain 1 (A) and strain 9a (B) grown at Fort Collins, Colo., in 1929. Strain 9a is susceptible to cercospora leaf spot, as evidenced by the dead outer leaves.

for genes determining major morphological characters. The plants of strain 9a used for seed production were sib progenies of the strain, each progeny forming a separate row. The plants of strain 1 were set in rows alternating with strain 9a. The seed produced was harvested separately from each row. In all, 11 seed lots were obtained, 6 from the various rows made up of strain 1, and 5 from the sister progenies of strain 9a (designated as 9a₁, 9a₂, 9a₃, etc.). These seed lots were used in test 1, and the results are given in the first section of table 2.

Each parental strain was distinct from the other. The difference in length and type of petiole, shape of leaf, and shade of green color,

associated with a peculiar habit of growth, gave a striking contrast in the foliar bouquet of the two strains (fig. 2). In populations arising from the reciprocal crosses, the foliage as well as the root type was used to distinguish between inbred and hybrid classes. All plants



FIGURE 2.—Field view of strains 1 and 9a and the hybrid $1 \times 9a$, grown at Fort Collins, Colo., 1932, showing greater foliage growth and vigor of the hybrid: A, Three-row plot of strain 9a (a) and of the hybrid $1 \times 9a$ (b); B, strain 9a (a), three-row plot of the hybrid $1 \times 9a$ (b), and strain 1 (c).

that were judged to have originated by selfing were eliminated; however, in case a plant could not be classified with sureness it was always included in the sample as a hybrid. The data as reported are considered to represent largely the performance of the hybrid class of the population.

The parental strains 1 and 9a had average root weights of 0.9574 and 0.9311 pound, respectively, indicating that under the conditions they were very similar in growth capacity. The hybrids 1 (rows 1, 3, 5, 7, 9, 11) \times 9a had an average root weight of 1.5271 pounds; the hybrids 9a (progenies 1-5) \times 1 had an average root weight of 1.5534 pounds. The striking vigor of the hybrid is shown by the comparison of average root weight of the 11 hybrid lots with the mean root weight of the two parents (fig. 3). The hybrid gave an average of 1.5391 pounds, corresponding to a calculated acre yield of 22.41 tons; and the parents, 0.9443 pound, corresponding to a calculated acre yield of 13.735 tons. When expressed as percentage of the mean weight

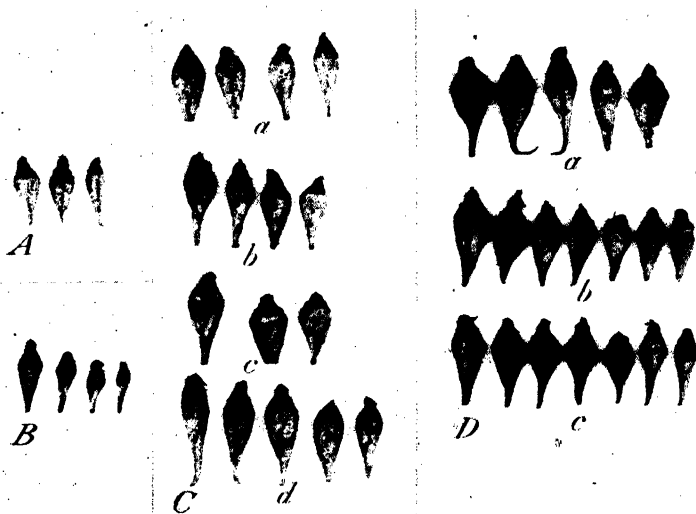


FIGURE 3.—Typical roots of the parental strains 1 (A) and 9a (B) and their hybrids, as obtained in test 1, Fort Collins, Colo., in 1932. C, Hybrid 1 \times 9a, roots grown from seed harvested from strain 1 as seed bearer; a, b, c, seed taken from rows 1, 3, 9, and 11, respectively. D, Hybrid 9a \times 1, roots from the reciprocal cross, in which sibs 2, 5, and 7 (a, b, c) of strain 9a were the seed bearers.

of the parents, the increase of the hybrid is approximately 63 percent. Since the parental strains were similar in yield, the hybrid exceeded each by approximately the percentage as found for the mean of the parents.

Previous tests had shown the parent strains to be distinctly unlike in richness of sucrose. The mean sucrose percentages obtained in this test, 14.05 for strain 1 and 11.10 for strain 9a, are in agreement with those obtained formerly. The sucrose percentages as shown in table 2 for this hybrid in all cases were higher than the low-sucrose parent, and in all but one case lower than the high-sucrose parent. The mean value for the hybrid approximated the mean of the parents. The hybrid populations in table 2 representing the cross 1 \times 9a, in which the strain higher in sucrose percentage was the maternal parent, were consistently higher in sucrose than the populations in the other group, resulting from the reciprocal cross, in which the

strain lower in sucrose was the seed bearer. The difference in sucrose percentage between the reciprocals is statistically significant. Bougy (1), in controlled crosses of sugar beets and forage beets (Vauriac), did not obtain perfect reciprocity for sucrose percentage. However, it seems justifiable to assign the difference in these comparisons of reciprocals to incomplete elimination of selfs, which would tend to shift the readings in the direction of the respective maternal parents rather than to postulate a specific maternal influence. Savitsky (5) reports that in crosses between varieties of high- and those of low-sucrose percentage the F_1 generation was, in this respect, close to the arithmetical average of the parents.

The total sucrose in the roots, which is the product of root weight and sucrose percentage, was markedly increased in the hybrid over that of either parent. Since the average sucrose percentage of the hybrid approximated the mean value of the parents, this increase may be attributed almost wholly to the greater root size of the hybrid. The percentage increase (67 percent) over the mean of the parents is about in the proportion of the relative root weights.

The increase in root weight assignable to hybrid vigor in this cross far exceeds the statistical requirement for significance. The difficulties of excluding all selfs have been discussed in connection with the sucrose percentage of the hybrid. It is to be noted that in the test of these reciprocal crosses seed produced from six groups or rows of strain 1 flanked by rows of sib progenies of strain 9a was used. There is a remarkable concordance of performance among these different representatives of the mating. The results show clearly that, regardless of the direction of the cross, the hybrid was significantly greater in weight than either parent. From this, it is concluded that the vigor of the hybrid is not merely the expression of dominance of some simple character possessed by one parent but is of the same nature as heterosis response demonstrated for other plants.

HYBRIDS INVOLVING MANY STRAINS

In tests 2, 3, 4, and 5, as shown in table 2, an attempt was made to include crosses that involved a wide range of parental types. Inbred strains known to possess important characters such as high sucrose percentage or disease resistance were more generally used in the pairings. To only a limited extent could reciprocal crosses of a given hybrid be included in the test, as deficiency in quantity or quality of seed obtained from one strain entering the cross commonly made omissions necessary. Crosses in which the seed produced by both parental strains was harvested as one lot were also included in the tests. Although seed lots obtained in this manner are not so serviceable for interpretation of hybrid vigor as those harvested separately for each strain entering a cross, their inclusion is considered of value because of the indication obtained of the type of performance to be expected if the two strains are interplanted for extensive seed production of a first-generation hybrid. The results of the 4 field tests, involving in all 41 hybrid populations, the respective parents, and the commercial brand, are given in table 2.

The data are first discussed with general regard to the hybrid vigor resulting from crosses between sugar-beet strains; then the response of individual strains when hybridized is considered because of indications given as to their possible breeding value.

ROOT WEIGHT OF HYBRIDS

As mentioned earlier, the comparison of the hybrid with its maternal strain, because of the incomplete elimination of selfs that can be effected at harvest, is probably the most conservative measure of performance. In such a comparison, however, as has been noted with other crop plants, the percentage increase in the yield of the hybrid over that of a low-yielding parent may be strikingly greater than the percentage found if the maternal parent itself is relatively high in yield.

In 30 of the 41 crosses the seed had been harvested separately for each strain entering a cross. The average root weights of the plants grown from hybrid seed were significantly greater than the average root weights of the respective maternal parents in 20 of the 30 crosses. In 10 cases significant differences between the hybrid and the maternal strain were not found. For the 30 comparisons the general average root weight showed an increase of 50 percent for the populations arising from these crosses over the general average root weight of the maternal parents.

The comparisons in terms of the mean of parents are also of interest, since all hybridizations can be included. In 31 of the 41 cases the root weights of the hybrids were significantly greater than the means for root weight of the appropriate parent strains; significant differences were not indicated in 10 cases, although in one cross 14b \times 10 the hybrid had been significantly superior to the maternal strain. The general average root weight of all hybrids exceeded the general average root weight of parents by at least 42.5 percent. In the consideration of the foregoing data, attention is again called to the fact that elimination of selfs was incomplete in many cultures.

The average root weight of the 41 hybrids was found to be 38.9 percent greater than that of the commercial brand, Pioneer. On the basis of extensive tests with commercial brands, among which Pioneer was included as a representative of sugar types, Skuderna et al. (7) found that as an average the sugar types were exceeded in root weight by yield types by approximately 10 percent. On such a basis it would seem that in acre yield of roots the hybrids as a whole compare very favorably with commercial yield types and that certain hybrids far exceed them.

SUCROSE PERCENTAGE OF HYBRIDS

For the 30 crosses in which the seed was kept separate as to the seed-bearing strain, it was found that in 17 cases the sucrose percentage of the hybrid was not significantly different from that of its maternal strain; in 2 cases it was significantly superior, and in 11 cases significantly inferior. The general average sucrose percentages for this entire group did not differ significantly from the mean of all strains involved as seed bearers.

The average sucrose percentage of the 41 hybrid populations, although slightly lower, did not differ significantly from the mean of the parental strains.

Twenty-three of the forty-one hybrids were not significantly different in sucrose percentage from the commercial brand, Pioneer. Nine were significantly less and nine significantly higher. The average sucrose percentage of the 41 hybrids equaled that of Pioneer.

This indicates, on the whole, a very satisfactory richness in sucrose for hybrid beets.

TOTAL SUCROSE IN HYBRIDS

Hybrid vigor was very pronounced in many of these crosses, increasing root weights significantly over the root weights of the parents. Since the sucrose percentages found approximated, as an average, the mean sucrose percentage of the parents, the total sugar produced was higher in the majority of cases than the mean sugar production found for the parental strains, this being assignable to the increased root yield of the hybrids.

Similarly, in the comparison of the hybrids as a whole with the commercial brand, since the average sucrose percentages of the hybrids and of Pioneer were approximately the same, sugar production of the hybrid populations exceeded that of the commercial brand by about the percentage found in the comparisons of root weight (39 percent).

RESPONSE OF INDIVIDUAL STRAINS WHEN HYBRIDIZED

STRAIN 1

Strain 1, which is discussed on page 722, occurred as a parent in 20 of the 41 hybrid cultures whose performance data are given in table 2. Field views showing one-row plots of various hybrids in which strain 1 occurred as a parent are shown in figure 4. In four instances the crosses were in duplicate. For the crosses with strain 2 and strain 9b, seed was available for test of reciprocals. The hybrid $1 \times 9a$, which occurred in test 2, has also been considered in the discussion of test 1.

In eight cases (tests 2, 3, and 4, table 2) seed was harvested from strain 1 as seed bearer. The performance data shown for these hybrids are based largely upon the F_1 portion of the progeny, since the rather distinctive selfs of strain 1 could be eliminated. For each F_1 progeny, the yield of roots is significantly above that of the maternal strain, above that of the pollen parent except in one instance, and above the mean root yield of the parental strains. The average percentage increase in root weight of the hybrids, found by averaging the appropriate individual percentages given in table 2, is 80.4 percent over the average root weight of maternal strains and 66.0 percent above the mean root weight of both parents. It also exceeds the average root weight of Pioneer by 60.3 percent.

Strain 1 occurs as pollen parent in seven crosses. The performance data for these are based upon mixed populations of hybrids except in the progenies from crosses $9b \times 1$ and 11×1 (test 2, table 2). Each of the seven progenies from hybridization gave a larger root yield than either parent, the increase in root yield over that of the respective maternal strain being statistically significant in four cases; the root yields were significantly greater than the yields of the pollen parent in 6 cases out of the 7, and accordingly exceeded the mean yields of both parents. The average percentage increases in root yield for these seven populations containing hybrids are 38 percent above that of both parents and 36.3 percent above that of the commercial brand Pioneer.



FIGURE 4.—Field views of one-row plots of two replications of test 2. Alternate plots planted with Pioneer to give common competition. A and B, Plots of D series: *a*, Strain 1; *b*, Pioneer; *c*, strain 16; *d*, Pioneer; *e*, hybrid 11×1; *f*, Pioneer; *g*, hybrid 16×11; *h*, Pioneer; *i*, hybrid 11×9a; *j*, Pioneer. C and D, Plots of E series: *a*, Hybrid 9a×11; *b*, Pioneer; *c*, hybrid 1×9a; *d*, Pioneer; *e* and *f*, strain 9a; *g*, Pioneer; *h*, strain 11; *i*, Pioneer; *j*, strain 1.

Strain 1 also occurred as a parent in five crosses in which the seed produced by the two strains entering a cross was pooled at harvest. The progenies obtained consisted not only of F_1 generations but selfs of each parental strain. Comparison of root weight with the mean root weight for the appropriate parent strains and for Pioneer can be made. In each cross, regardless of inclusion of selfs, the populations obtained were significantly superior in root yield and in sugar production to the mean of parents and to Pioneer, the average percentage increase being, for each basis, approximately 34 percent. Superiority in sugar production was in about the same proportion.

In general, the result of crossing strain 1 with 15 unrelated sorts was a marked increase in vigor in the first-generation hybrid (fig. 5, *A, B, C, D*). The preponderance of evidence indicates reciprocity of the effect of hybridity when reciprocals are compared, or when the yields of hybrids in which strain 1 is the pollen parent are considered in terms of root yields of the respective mother strains.

STRAIN 2

Strain 2, whose pedigree shows six generations of selfing, interspersed with 4 years of group increase, has been characterized by heavy foliage production and exceptionally high root yields. It has shown moderately high sucrose percentage and moderate leaf spot resistance. In test 3 (table 2) it was used only in crosses with strain 1. Data are available for this cross from progenies of seed harvested from each parent as a seed bearer, and from a progeny in which seed from both parents was pooled.

In the cross 1×2 , the hybrid was superior in performance to either parent. In the reciprocal, a gain over the maternal parent was shown, but this, however, did not reach significance. The hybrid progeny grown from the pooled seed gave an acre yield of roots of 16.96 tons. The mean acre yield of roots of the reciprocals was 17.09 tons, an entirely concordant value, which exceeds significantly the mean root yield of the parents.

The divergence in root yields in the reciprocals seems attributable to difference in harvest methods employed. In the plots of the cross 1×2 , it was possible to exclude selfs rather rigorously, while for the plots of the reciprocals samples taken were not so restricted. The hybrid 2×1 did not exceed significantly the root weight found for strain 2. Since strain 2 is not known to be strongly self-fertile, it is to be presumed that the progeny contained a fair proportion of hybrids. It is known for other crops that the heterosis effect is less strikingly shown by vigorous inbred strains.

STRAIN 3

Strain 3 is characterized by dark-colored foliage, medium root weight (about equal to that of Pioneer), and high sucrose percentage. In leaf spot resistance it ranks above strain 2, but is less resistant than strain 1. Although it has since been extensively used in crossing, in these tests it was represented as a cross with strain 1 (test 5, table 2) in which the seed was not kept separate as to seed bearers (fig. 5, *C*). The performance of this hybrid indicated a root yield 40 percent above the mean of the two parents; the hybrid apparently is significantly above Pioneer in root yield. Sucrose percentage was high and approximated that of strain 1.



FIGURE 5.—Sugar-beet roots from plots planted with parental strains and with seed obtained by interplanting strains. (Roots taken from border rows of the plots but representative of roots from which data were obtained.) A, Strain 5a (a), hybrid 1×5a (b), and strain 1 (c); test 4. B, Strain 7 (a); hybrid 7×1 (b), strain 1 (c); test 3. C, Strain 1 (a), hybrid (1×3) (b), and strain 3 (c); test 5. D, Strain 1 (a), hybrid (1×6) (b), and strain 6 (c); test 5.

Strain 4 is represented by separate substrains, a and b, having essentially the same pedigree and close similarity in habit. The strain has been under observation since 1926 because of strong resistance to *Cercospora beticola*. Strain 4b was used as the seed bearer in crosses with strains 1 and 15. The hybrid 4b \times 1 did not give a root yield significantly above that of the mother strain; the root yield of the hybrid was apparently superior to the mean of the two parents. The hybrid 4b \times 15 gave a root yield which was significantly above that of either parent. In the latter cross, no attempt was made to separate the progeny into hybrid and selfed classes, nevertheless the root yield attributed to the hybrid was 43 percent above that of substrain 4b and 37 percent above the mean root yield of the parent strains. The root yield of Pioneer was exceeded by the hybrid by 55 percent. The occurrence of an epidemic of cercospora leaf spot has been mentioned previously. In these tests, the hybrid 4b \times 15 was strikingly resistant, whereas Pioneer was susceptible. The ratio of performance with respect to Pioneer was decidedly influenced by the differences in disease reaction. However, the disease influence was not a factor of importance in the comparative vigor of the cross and parents, since the strains crossed are among the most resistant inbreds in the test. Strain 4a was crossed with strain 5b and strain 12, respectively, the seed from the seed bearers being pooled in the case of each hybridization. In the cross 4a \times 5b, the root weight of the hybrid was only slightly above the mean root weights of the strains entering the cross or of Pioneer. The hybrid 4a \times 12 was found to produce a root weight not significantly superior to the parental mean for root weight (fig. 6, A). It exceeded the commercial brand in root weight by 37 percent.

STRAIN 5

Strain 5, which is characterized by strong cercospora leaf spot resistance and a high degree of uniformity, was used as substrains a and b, which differed slightly in pedigree but represented selections out of the same genetic complex. Seven crosses were made using strain 5 (either a or b) with strains 1 (in duplicate), 4a, 8b, 9c, and 10 (reciprocal). The hybrids 1 \times 5a and 4a \times 5b have already been discussed. In performance, the hybrids were somewhat inconsistent, which may indicate that strain 5 is restricted in its value as breeding material. In the case of the cross with strain 10, in which reciprocals were tested, root yields differed rather widely but probably not significantly. The hybrid 10 \times 5a exceeded Pioneer significantly in root yield but not its parents. The hybrid 5b \times 9c exceeded in root weight the mean for root weight of the parents and significantly out-yielded the commercial brand, Pioneer, as well.

UTILIZATION OF STRAINS 1 TO 5

A detailed discussion has been given of strains 1 to 5 and their behavior in hybridizations because these strains, on the basis of this

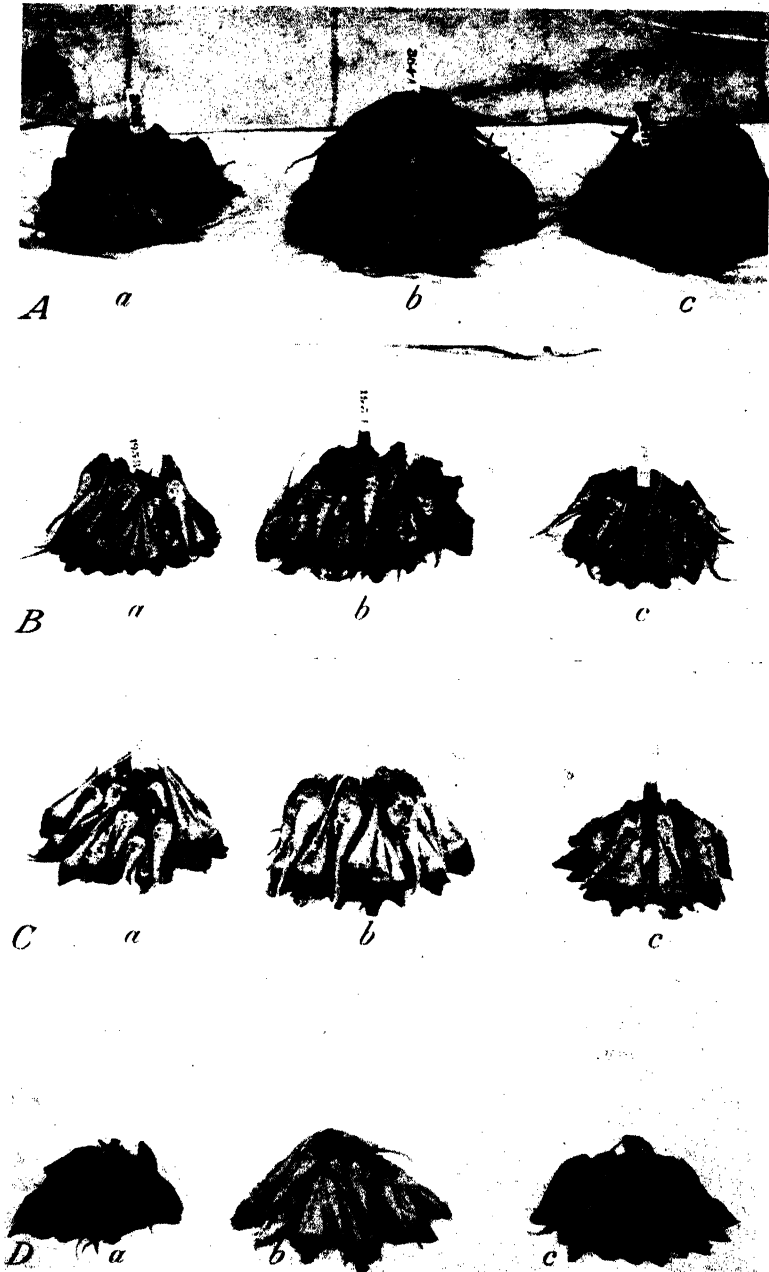


FIGURE 6.—Sugar-beet roots from plots planted with parental strains and with seed obtained by interplanting strains. (Roots taken from border rows but representative of populations from which data were obtained.) A, Strain 4a (a); (4a×12) (b), and strain 12 (c). B, Strain 7 (a), hybrid 8a×7 (b), and strain 8a (c). C, Strain 8b (a), hybrid 8b×14b (b), and strain 14b (c). D, Strain 11 (a), hybrid 11×Pioneer (17) (b), and Pioneer (c).

test and other information, have been used in the production of a synthetic variety released for grower use as U. S. 217. A brief report has been made on this variety under the designation Accession 217 (2); the strains of which it is composed were referred to as a, b, c, d, and e, corresponding to the numbers 1 to 5 as used here. U. S. 217 has performed satisfactorily in yield and in sucrose percentage, exceeding commercial brands used as checks. It has shown definite leaf spot resistance.

OTHER STRAINS

As shown in table 2, strains 6, 7, 8 (fig. 6, *B* and *C*), and 10 have given some hybrid combinations that show significant gains attributable to hybrid vigor. Strains 14 and 15, which were used only as pollen parents, have given evidence of inducing heterosis response and are discussed in connection with the various strains with which they were combined. Strain 12 was crossed with strains 1 and 4a, the seed not being kept separate as to seed-bearing strain. In the cross with strain 1, the root yield was significantly above the mean yield of the parents. In the cross with strain 4a, significant increase was not shown. Strain 13 was crossed with strain 6, the seed not being kept separate as to seed bearer. The root yield of the hybrid was not significantly above the mean yield of the parents.

Strains 9 (including its substrains a, b, and c), 11, 16, and the commercial brand, Pioneer, were used in crosses and have given additional information on hybrid vigor, to which attention is called.

Strain 9a as mated with strain 1 was extensively used in test 1. The hybrid $1 \times 9a$, representing the same cross as used in test 1, and the hybrid $1 \times 9b$ and its reciprocal were included in test 2. Because of the close similarity of the substrains, these hybrids are entirely comparable; limited seed quantities, however, made it necessary to omit them from the first test. The root yields as found in test 2 for the hybrid of strain 9 and strain 1 showed for these three cases increases over the mean of the parents of 111, 89, and 84 percent, respectively. If comparisons are based on a mean yield for strain 9, obtained by averaging the root yields of 9a and 9b, this value for strain 9 being in turn averaged with the root yield found for strain 1, the percentages for increase of hybrid over the parental means become, respectively, 201, 199, and 194. In terms of the performance of the commercial brand, the gains in root yield for the three cases are, respectively, 72, 70, and 60 percent. The mean acre yield of roots, 20.19 tons as calculated for the hybrid in test 2, is somewhat less than the average shown in test 1, 22.41 tons; in the two tests, the commercial brand shows comparable differences in its yields. The two tests are in full accord as to the relatively high hybrid vigor shown.

Strain 11, used as a parent in crosses, affords a very interesting series of hybrids for study of heterosis response. The strain is characterized by having a pink epidermis on the root and crown as well as on the basal portions of the petioles, these color characters being dominant. When strain 11 was crossed with strains without pink roots or petioles a definite marker character to identify the F_1 plants was available in the crosses in which strain 11 was the pollen parent. Thus, in the cross $9a \times 11$, all plants with pink roots or petioles were identified hybrids, and the performance data shown for this cross (test 2, table 2) are based on the F_1 class (fig. 7). The increase in root



PLANT 104



FIGURE 7.—*A*, Hybrid 9a \times 11. View along the row of a plot planted with seed obtained by interplanting strains 9a and 11, the former being the seed bearer. The ruler stands between two selfs of 9a, the plants adjacent are F₁, their hybrid nature being assured by pink petiole color, a dominant character transmitted by strain 11. *B*, Hybrid 9a \times 11. Roots from a border row of another plot planted with some of the same seed as the plot shown in *A*. The roots are arranged as they occurred in the row. The five large roots are from F₁ plants, as identified by the pink petiole character. *C*, *a*, Strain 11, as grown in nearby plot. *b*, Hybrid 11 \times 9a. Roots grown in border rows of a plot planted with seed in which strain 11 was seed bearer; roots could not be closely separated into hybrid and selfed classes. These are representative of roots from which data were obtained. *c*, Strain 9a as grown in nearby plot.

yield for the hybrid is more than double that of either parent. In the reciprocal cross, the performance record could not be based entirely on the F_1 class, although the majority of selfs could be eliminated. In spite of this limitation, the performance of the hybrid was outstanding.

The cross 11×1 (test 2, table 2) is another example of striking hybrid vigor. It should be noted that strain 11 is very susceptible to cercospora leaf spot. If strain 11 is mated with a resistant strain, for example the cross of strain 11 and strain 1, the hybrid vigor that may be indicated on the usual basis of interpretation is undoubtedly influenced by the unlike effect of the disease on the parental strains. Since resistance to the disease in this cross is at least partially dominant over susceptibility, the hybrid would give relatively a high performance in relation to the more susceptible parent in tests conducted under disease epidemics. It is difficult therefore to assess the extent to which the reaction of disease was a factor in the performance of the crosses of strain 11 with strains 9 or 1. It is certain, however, that the disease factor was not sufficient to account for the striking contrast of hybrid and parents.

Strain 11 as a parent in crosses with strains 1 and 9a had produced hybrids remarkably superior to the parents, yet when crossed with strain 16 it did not produce a similar response. In the cross 16×11 (test 2, table 2), the performance of the certified hybrid plants was not appreciably unlike that of the mother strain. Reference to table 1 indicates that strain 16 is removed from the open-pollinated variety by only two generations, one of which was a selfed generation. The heterozygous condition of strain 16 may account for the results obtained.

Selected roots from the commercial brand, Pioneer, were crossed with strain 11 and with strain 1 to produce the type of cross usually designated as top cross. In the cross Pioneer $(17) \times 11$ (table 2), it was possible to base the performance record upon hybrid plants by use of the red-petiole-color character of strain 11, which is not found in Pioneer. In the reciprocal cross, the plot sample could not be reduced so positively to F_1 plants (fig. 6, *D*). In the case of both Pioneer $(17) \times 11$ and the reciprocal, the hybrid yield was about the same as that of Pioneer. The cross Pioneer $(17) \times 1$, and the reciprocal (table 2, tests 1 and 2) show performance records in line with the top cross just discussed.

It may be significant, however, that using inbred strains that are low in yield in top crosses did not result in a hybrid reduced in vigor below the average of the heterozygous variety. Hence, it may be possible by top crossing to impart desirable characters from highly resistant or high-sucrose inbreds to vigorous heterozygous varieties without reduction of yielding capacity taking place.

DISCUSSION AND SUMMARY

The hybrids obtained in more than two-thirds of the crossings reported in these tests have shown significant increase in root weight attributable to vigor of hybridity. With some hybrids no response was shown, and in others the gain found was within chance occurrence. In general, these quantitative effects from hybridization are believed indicative that heterosis is a determinative factor in root yields of sugar beets.

In certain crosses, the hybrid plants were definitely identified by a color character, and in the sampling of plots selfs could be excluded. In one case, the root yield of the hybrid was more than double that of either parental strain. In the cross 1 \times 9 and its reciprocal, the determinations were based largely on F_1 plants, since the selfs could be eliminated fairly completely from the plot samples. The hybrid was given extensive test; and it was shown that, regardless of the direction of the cross, the hybrid was significantly greater in root weight than either parent by more than 60 percent. From this, it has been concluded that the effects demonstrated are attributable to heterosis and are not the expression of some simple dominants possessed by one parent. Crosses involving strain 1 as a maternal parent and various other inbreds as pollen parents were consistent in giving hybrids superior in root weight to strain 1. In the crosses in which strain 1 occurred as the pollen parent, the hybrid was often, although not always, superior to the maternal strain. This seemed more likely to occur when strain 1 was mated with vigorous strains.

In the cases in which no attempt was made to separate hybrid and selfed classes in the progeny obtained from a pairing, the performance found was definitely influenced by the presence of lower yielding selfed plants, bringing about a strong trend in the yields toward that of the seed-bearing parent. In spite of this effect of nonexclusion of selfs, many records were obtained in which the progeny from a mating, taken as a whole, was significantly superior to the mean of the parents and frequently superior to each parent entering a cross.

Nearly three-quarters of the hybrid progenies gave root yields 25 percent higher than were found for the commercial brand used in each test. Thus, 7 hybrids out of 10 used in test 2 (table 2), 10 out of 11 in test 3, 6 out of 11 in test 4, and 7 out of 9 in test 5 were significantly superior to Pioneer in root yield. The commercial brand was, in many cases, somewhat more seriously affected by cercospora leaf spot than the more resistant strains or their hybrids. Because of this factor, direct inference as to the effect of hybrid vigor in increasing root weight may not be made, but it seems clear that with certain hybrids the gains in yield over the commercial brand are enough beyond those reasonably attributable to superior disease resistance to indicate definite increase in productivity from heterosis.

Coordinate in importance with the effect of hybridity on root weight is its effect on richness in sucrose. Reciprocal crosses (test 1, table 2) seemed to show a trend in sucrose percentage toward that of the maternal parent. This is believed assignable to incomplete elimination of selfs, since the mean sucrose percentage of reciprocals was approximately equal to the mean sucrose percentage of the parents. In the tests of 41 hybrid progenies, their mean sucrose percentage rather closely approximated the mean sucrose percentage of Pioneer, indicating strong maintenance of requisite quality.

It is customary to evaluate sugar-beet varieties in terms of gross sugar produced per unit of area. Because of the definite indications for many of the hybrids of strikingly increased weights over the parental material and of sucrose percentages about equal to the mean of parents, the increase in productivity in terms of sugar follows essentially the response in root weight attributable to hybrid vigor.

The situation as to the yielding capacity of inbred strains is in a measure shown by these tests. Among the 16 strains used, some of

which are the product of many generations of inbreeding, 2 were found, under the conditions of these experiments, to be significantly superior and 2 significantly inferior to the commercial brand in acre yield of roots. The other strains did not differ significantly in root yield from Pioneer. The average performance of the inbred strains and that of the commercial brand were very similar. Certain strains were strikingly resistant to cercospora leaf spot, while others were susceptible. As a group, they were more resistant than the commercial brand, a factor which must be taken into account in consideration of relative yielding capacity. The performance of many of the inbred strains as they now exist warrants the opinion that their productivity is still relatively high after a number of generations of inbreeding.

The experience with first-generation hybrids suggests that in the program for sugar-beet improvement purposeful crossing of strains found to give strong heterosis response may have an important place. Introduction of a synthetic variety, U. S. 217, produced by intercrossing five of the strains which were found in these tests to give indications of such response and which were, in addition, high in cercospora leaf spot resistance, represents an attempt in this direction.

The practical breeding problems to be faced in production of high-yielding hybrids or desirable synthetic varieties hinge in part upon the production of disease-resistant and otherwise desirable inbred strains. Advantageous combinations probably will need to be found because of the general tendency of yielding capacity to decrease with inbreeding. Seed setting among the strains has not been excessively low, whence it would appear that seed stocks of these strains in considerable size may be produced without excessive cost. The important problem of securing a high degree of intercrossing between chosen strains must be faced. The only practical method now available of securing intercrossing seems to be to pool the seed of strains for planting the seed field. In the production of U. S. 217, the five inbred strains were variously paired to produce stock seed in three fields, and the stocks thus obtained were pooled for use as planting stock for commercial seed production by the field-overwintering method. A study of the synthetic variety thus produced indicates that a fairly high degree of intercrossing took place, but many apparent selfs were found. Observations made in the course of these tests with single crosses showed that the amount of cross fertilization occurring may vary from as much as 90 percent to less than 10 percent. Further, it is known that climatic conditions affect the rate of seed-stalk formation, seed setting, and viability of strains differentially, making it necessary that response of strains to these factors be taken into account in the sugar-beet-breeding program.

Certain crosses also indicate that heterozygous varieties otherwise desirable may be improved with respect to specific characters by the method of top crossing. By a course of intensive inbreeding and selection in segregating populations, it is possible to make faster progress in establishment of characters such as high disease resistance or high sucrose percentage than will be accomplished by mass selection. In the event that reduction in vigor takes place, it appears from these tests that the inbred strains which can contribute a desired character may be used in top crosses without necessarily reducing the growth potentialities of the heterozygous mother.

Methods employed by European firms engaged in production of sugar beet seed are essentially mass-selection methods. A brand or variety as supplied consists of a heterozygous population which, as a result of adherence to progeny tests in the selection of breeding stock, conforms in average performance with reasonable fidelity to a given physiological classification, such as tonnage, intermediate, or sugar type. As might be expected, improvement has been slow. That there has been increase in productivity in recent decades has been questioned (3). Varieties with special adaptation have, in general, not been produced. In many respects, the European varieties of sugar beet are comparable in status to high-grade open-pollinated corn varieties. The evidence that strains of sugar beet can be produced which have special characteristics such as disease resistance or high sucrose percentage and the showing that definite heterosis response exists between certain strains opens up definite opportunities in development of varieties better adapted for American conditions and actual increase in sugar beet productivity.

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ROOT RESPONSES OF NONINFECTIOUS HAIRY ROOT APPLE SEEDLINGS UNDER DIFFERENT METHODS OF PROPAGATION¹

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INTRODUCTION

In the last decade the commercial production of apple (*Malus*) seedlings for use as rootstocks has greatly increased in the region around Yakima, Wash. During this period seedling growers, especially in this region, have been using in large amounts domestic American seed obtained from various commercial apple varieties of or the Northwest.

In cooperative experiments with several nurserymen, the writers have had an opportunity to compare seedlings grown from imported French crab with those grown from domestic seed. It has been observed repeatedly that a relatively large percentage of the seedlings from domestic seed are characterized by a condition on their roots that has been termed "hairy root" (28)² and "simple form hairy root" (11, 17). The latter term was used to describe 1-year-old seedlings that exhibited excessive lateral roots in the region extending from the collar to a distance of several inches below the ground line.

In conversation and correspondence with nurserymen the writers have referred to this type of malformation as "noninfectious hairy root," because no evidence that the condition is the result of infection has been noted either in the literature or in field observations.

The situation resulting from the use of domestic seed has created a problem of considerable economic importance to the seedling grower and to the commercial propagator. Field counts indicate that approximately 20 percent of the average run of domestic seedlings are affected. The subject is also of interest to the investigator concerned with the problem of rootstocks, because many of the clones of the Malling types of Paradise and other dwarfing apple stocks as selected by Hatton (5, 6) exhibit root systems that show conditions somewhat similar to noninfectious hairy root. The extent of this similarity, however, cannot be determined until comparative observations are made on 1-year-old plants of these Malling clones.

The studies reported herein were undertaken to obtain information on the nature of the root systems of these abnormal seedlings and their responses under different methods of propagation. Specifically information was needed as to what advice should be given to nurserymen regarding the use of normal-appearing portions of affected seedlings.

¹ Received for publication February 7, 1940.

² Italic numbers in parentheses refer to Literature Cited, p. 753.

REVIEW OF LITERATURE

In Downing's time (4), the fact that root systems differed, especially in the effect on the scion variety, was well recognized. He noted the dwarfing effect produced on apple and cherry when worked on Paradise and "perfumed" (mahaleb) roots, respectively. Paradise and Doucin stocks were imported into this country for many years specifically for producing dwarf trees (18, 35). In general, these stocks were characterized by aerial burrknots and by masses of roots growing in tufts or clusters near the ground line. Their rooting habits and tendency to produce shoots near the ground line rendered them especially adaptable to propagation by "stooling."

Knight (13) and many others have referred to the use of burrknots as an aid to rooting. The practical synonymy of the terms "burrknots," "aerial crown gall," "stem tumors," etc., has been noted (17, 27, 30), and despite the fact that symptoms have been produced by inoculations (27), the evidence indicates that these malformations are not caused by a pathogenic organism but are of a genetic nature (9, 10, 16, 17, 20, 29). That individual plants exhibiting burrknots are characterized generally, if not invariably, by abnormal rooting is indicated by the illustrations of several investigators (5, 6, 11, 17, 22) and by general observations.

The term "hairy root" (28) was used to describe the large masses of roots on the main root axes of nursery apple trees. Later, Hedgcock (11) used the term "simple form hairy root" for these and similar malformations on the stem and roots of apple seedlings. He described the symptoms as consisting "of numerous small roots growing out at nearly right angles, either singly or in tufts, from an older root or stem." In the absence of information as to the cause of this condition, he naturally advocated segregation of affected seedlings as a routine precautionary measure. However, it is significant that he concluded (1) that the condition was not associated with wounding, (2) that it was practically eliminated when affected seedlings were not used in grafting, and (3) that when affected seedlings were used in piece-root grafting there followed in the second year "an atrophy of the hairy root pieces."

As a result of experiments in which apple seeds were grown in steamed soil, Muncie (21) concluded that "the fibrous type of hairy root is probably not infectious" and that the condition is the result of some inherent character of the seedling. Later, however, Muncie and Suit (22) showed a seedling with the "fibrous type of hairy root" as "characteristic of the condition found on nursery trees grown in sandy soil." They stated that on the quince the formation of clusters of roots appears to be the natural rooting habit and is not associated with bacterial infection. Maney (17) planted several hundred 1-year-old affected apple seedlings and noted that the masses of rootlets that subsequently originated on the stem arose "from typical burrknots." He concluded that "the simple form of hairy root on apple seedling stock is caused by burrknot."

The problem of adequately describing various types of root systems is obviously a difficult one. That individual roots and root systems as a whole or in part may exhibit quantitative and qualitative differences is well recognized (5, 8, 14, 23, 24, 26, 32, 33). Criteria

for descriptions, however, are wanting (31), and as a result considerable confusion exists. The various interpretations that have been placed on the word "fiber" illustrate this point. Other difficulties involved include variations due to the many environmental factors that may be encountered and to the fact that different parts of the root system often display unlike characteristics.

Under the existing situation, therefore, emphasis in this report will be placed on quantitative features and, as a matter of expediency, root systems that exhibit a comparatively large number of small lateral roots on the current season's growth will be designated as abnormal (affected). Although there is no sharp dividing line between the normal and abnormal, nevertheless symptoms of abnormality as they refer to excessive numbers of lateral roots originating on a well-defined axis, can be roughly classified according to degree, as slight, moderate, or pronounced.

MATERIALS AND METHODS

The experiments were performed with the use of (1) root cuttings, from which plants were propagated directly; (2) grafts, made from piece-root cuttings; (3) 1-year-old seedlings; and (4) seed from various sources. Plantings were made either in steamed soil in the greenhouse or in nontreated soil in the field.

Seedlings showing abnormalities in roots or in rooting are designated "noninfectious hairy-root seedlings," and the symptoms are classified as slight, moderate, or pronounced, on a quantitative (numerical) basis. The parts of these affected seedlings not exhibiting symptoms are referred to as "clean" to distinguish them from similar-appearing areas on normal plants.

EXPERIMENTS AND RESULTS

PRELIMINARY EXPERIMENTS

Because of the difficulty of classifying seedlings that exhibited very slight symptoms of noninfectious hairy root, several hundred 1-year-old seedlings that were classed as "borderline" cases were planted in 1920 to observe their future development. When dug 1 year later, approximately 50 percent of these seedlings showed definite symptoms. This experience was of value in subsequent experiments, since it afforded some basis for diagnosis in discarding seedlings intended for use in crown gall and hairy root experiments.

In preliminary experiments in 1936 a small number of root cuttings were made from the seedling (domestic) roots of several 1-year-old grafts that showed moderate symptoms of noninfectious hairy root. The cuttings were planted horizontally about 1 cm. deep in sterile soil in a greenhouse and grown in comparison with root cuttings obtained from grafts of a clonal material known as U. S. D. A. Vt. 323, which had been under observation for several years and which had never shown symptoms of abnormal rooting. When examined 1 year later the U. S. D. A. Vt. 323 root cuttings were normal; the six surviving plants of the moderate noninfectious hairy root series showed relatively slight symptoms on the original root piece, but pronounced symptoms at the base of the new shoot growth.

In the same year, root cuttings from the "clean"-appearing areas of noninfectious hairy root seedlings were planted. All of the 12 surviving plants showed symptoms.

The results of these preliminary experiments with root cuttings indicated that although the condition is systemic or inherent, stem rooting tends to reduce the severity of the symptoms on the original root piece. Accordingly, the experiments of 1938, reported subsequently, were designed to furnish additional evidence along these lines.

Included in these earlier experiments is a report of the results obtained in comparisons of seedlings grown from seed procured from various sources. Field observations had indicated that the progeny of domestic seed were particularly affected. The data in table 1 show wide variations in the percentage of noninfectious hairy root seedlings in the progeny of certain crosses and in the seedlings from certain seed sources.

TABLE 1.—Amount of noninfectious hairy root observed on 1-year-old seedlings segregated as to source of seed

Year	Source of seed	Locality where grown	Seedlings examined	Noninfectious hairy root seedlings
			Number	Percent
1936	Domestic.....	Beltsville, Md. (steamed soil, greenhouse).	54	20
	French crab.....	do	49	2
	Domestic.....	Beltsville, Md. (field).	520	16
	French crab.....	do	322	4
1937	Domestic.....	Kansas.....	300	16
	French crab.....	do	300	2
	Delicious.....	Beltsville, Md. (field).	200	2
	Rome Beauty × O ¹	do	200	5
	Winesap × O.....	do	200	5
	French crab.....	do	200	0.5
	Baldwin × O.....	Beltsville, Md	35	0
	Ben Davis × O.....	do	350	16
	Bonum × O.....	do	147	21
	Delicious × O.....	do	244	5
	Domestic × O.....	do	50	16
	Gallia × O.....	do	204	14
	Grimes Golden × O.....	do	87	13
	Golden Delicious × O.....	do	220	18
	Jonathan × O.....	do	136	0
	King David × O.....	do	70	0
	Macoun × O.....	do	41	22
	McIntosh × O.....	do	170	13
1938	McIntosh × Delicious.....	do	151	30
	McIntosh × Northern Spy.....	do	219	24
	Nero × O.....	do	100	0
	Northern Spy × O.....	do	342	32
	Northwestern Greening × O.....	do	220	9
	Oldenburg (Duchess) × O.....	do	63	0
	Rome Beauty × O.....	do	225	15
	Stayman Winesap × O.....	do	53	5
	Starking × O.....	do	164	4
	Stella × O.....	do	105	2
	Summer Pearmain × O.....	do	102	12
	Transcendent × O.....	do	20	0
	Wealthy × O.....	do	100	0
	Williams × O.....	do	165	0
	Winesap × O.....	do	108	17
	Winter Banana × O.....	do	299	9
	Yellow Transparent × O.....	do	35	0
	York Imperial × O.....	do	191	22

¹ "× O" signifies open or unprotected pollination.

EXPERIMENTS WITH GRAFTS IN 1938

Forty-three seedlings showing symptoms of noninfectious hairy root ranging from moderate to pronounced were selected, and each

seedling was given an individual number and thus identified as clonal material. These seedlings showed excessive rooting, extending from the hypocotyledonary region at the ground line to a distance ranging from 2 to 4 inches down the main axis. Typical examples are shown in figure 1, *A*, *B*, *C*, in comparison with a normal seedling (*D*). The



FIGURE 1.—One-year-old apple seedlings typical of those used in the experiments: *A*, *B*, Seedlings showing moderate symptoms of noninfectious hairy root; *C*, seedling showing somewhat less symptoms; *D*, normal seedling.

lower portions of the main axis and most of the side roots of these noninfectious hairy root seedlings appeared normal in all respects; such normal-appearing areas are designated "clean."

Stem cuttings (scions) were grafted to the proximal root pieces of the seedling from which they were cut, thus preserving the original combination of each graft. Of these 43 grafts that were planted in the

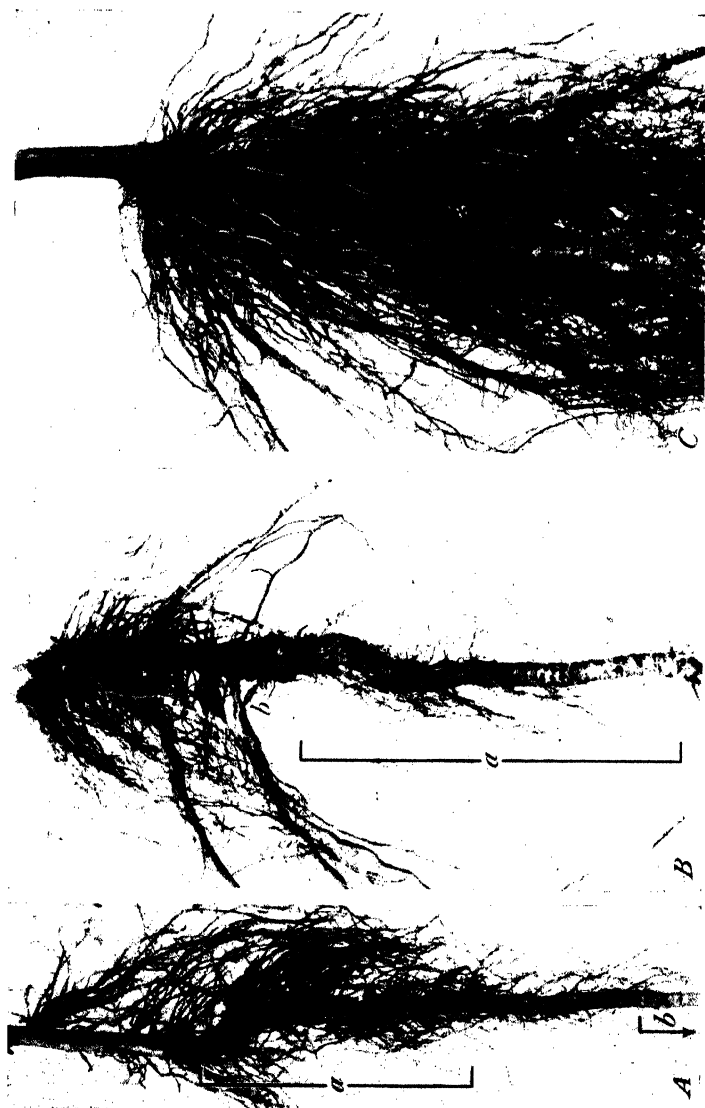


FIGURE 2.—Results obtained in the grafting and root-cutting experiments. *A*, The original 1-year-old seedling shows moderate symptoms. The region designated at *a* was used in the piece-root grafting a scion taken from the stem of this seedling. The "clean" area, *b*, at the distal end of this seedling, was used in the root-cutting experiment. *B*, 1-year-old tree resulting from grafting a scion from the seedling shown in *A* to the root piece shown in *A* at *a*. The scion has rooted and the symptoms on the original root piece *a* have been suppressed. The union is at *b*. *C*, 1-year-old tree resulting from growth of root cutting made from the "clean" (distal) region of seedling shown in *A*, *a*. There is no stem rooting, and the symptoms on the root are pronounced.

field, 40 grew and were examined as 1-year-old trees. The results (table 2) show that the symptoms on the original root pieces of the 1-year-old grafts were in all cases less pronounced than when they were originally grafted, as judged by comparison with photographic records. In all cases slight to pronounced rooting occurred on the scion; the more pronounced the scion rooting, the more the symptoms were suppressed on the original root piece. As an example, the condi-



FIGURE 3.—A 1-year-old graft resulting from grafting a scion, from a seedling showing pronounced symptoms, to its own root piece. Pronounced symptoms are apparent on the scion part of the graft, but the symptoms on the original root piece (*a*) have been suppressed. The side shoot (*b*), originating just below the graft union, exhibits pronounced symptoms at the region near the ground line.

tion of the root in the original seedling is shown in figure 2, *A*; in the 1-year graft in *B*; in the plant that grew from a root cutting from a "clean" portion near the distal end of this seedling in *C*. In this illustration the symptoms on the original seedling now incorporated in the 1-year graft are classed as slight. As might be expected, the underground parts of adventive shoots that occasionally sprouted from below the graft union and subsequently rooted exhibited pronounced symptoms at the distal regions (fig. 3).

TABLE 2.—Results obtained on grafts whose root pieces showed moderate to pronounced symptoms of noninfectious hairy root when grafted

Symptoms on original root pieces of 1-year-old seedlings	Grafts that grew	Symptoms on the 1-year-old trees					
		On original root piece			On scion		
		Slight	Moderate	Pronounced	Slight	Moderate	Pronounced
Moderate	Number 21	Number 21	Number 0	Number 0	Number 6	Number 12	Number 3
Pronounced	19	0	19	0	5	9	5

EXPERIMENTS WITH ROOT CUTTINGS IN 1938

Seventy-eight piece-root cuttings were taken from the same 43 noninfectious hairy root seedlings the proximal root pieces of which were used for grafting in the preceding experiment. These root cuttings were classified either as "clean" or with respect to the degree of symptoms displayed. The cuttings were segregated according to the seedlings from which they were obtained. They were planted vertically in pots in steamed soil with the top ends partly above ground so as to prevent rooting from the new shoots. Forty-four of these root cuttings produced plants that were dug as 1-year-old trees and classified as outlined in table 3.

TABLE 3.—Results obtained from planting root cuttings taken from noninfectious hairy root seedlings

Symptoms on root cutting	Cuttings that grew	Symptoms on the 1-year-old plants			
		Slight	Moderate	Pronounced	Moderate to pronounced
	Number	Number	Number	Number	Number
"Clean" (no symptoms)	32	2			30
Slight	2	0	2	0	
Moderate	8	0	6	2	
Pronounced	2	0	0	2	

The results show that under suitable conditions the "clean" (symptomless) parts of affected seedlings will develop symptoms. In the absence of scion rooting, the symptoms on the original root pieces either were made manifest on plants from the 32 "clean" cuttings, or were in general accentuated on plants from the affected cuttings, as determined by comparison with the photographs of the original roots (fig. 2, A, b; C).

For comparison, 50 seedlings that appeared normal were selected for root-grafting and root-cutting experiments. A piece of the stem from each seedling was grafted on the proximal part of its own root. When dug 1 year later the root systems of 47 of the grafted trees were normal; the remaining 3 showed moderate symptoms of noninfectious hairy root on the original root piece. In the lot of 47 trees, scion rooting invariably occurred; but these scion roots generally occurred singly, and from their size and texture they were tentatively considered as normal (fig. 4, A).

Of the 100 root cuttings from the 50 seedlings selected as normal, 56 grew. The root systems of 53 of the resulting 1-year-old plants were classed as normal (fig. 4, B); 3 exhibited moderate symptoms of noninfectious hairy root.

The fact that a few of the plants grown from parts of normal seedlings evidenced symptoms of noninfectious hairy root was somewhat surprising. The percentage is considerably higher than that usually encountered in routine grafting experiments made by the writers.



FIGURE 4.—Trees resulting from the use of normal seedlings. *A*, A 1-year-old graft resulting from grafting a scion from the seedling to its own root piece. The lower part of the graft union is at *a*. Despite the scion roots, which are considered normal, the caliper of the root piece is very good. *B*, A 1-year-old tree resulting from a piece-root cutting taken from a normal seedling. Note the branching habits of the numerous smaller roots.

It is possible that rooting above the collar may have masked noninfectious hairy root symptoms on these seedlings when they were selected.

EXPERIMENTS WITH 1-YEAR-OLD SEEDLINGS

In addition to the experiments with grafts and root cuttings, twenty-five 1-year-old seedlings showing moderate symptoms were cut back to approximately 10 inches of stem growth and were planted horizontally to encourage rooting from the buried stem tissue. Of the 23 plants surviving 1 year later, 21 had rooted from the stem, and the noninfectious hairy root symptoms on the original root were suppressed in direct proportion to the amount of stem rooting. These stem roots all came from the regions of the buds, usually in clusters of several to

many. The plants shown in figure 5 were selected to illustrate various types of stem rooting and to show the lack of symptoms on the original roots, which had exhibited moderate symptoms when planted. The plants shown in figure 5, *A* and *B*, were typical of the lot; that shown

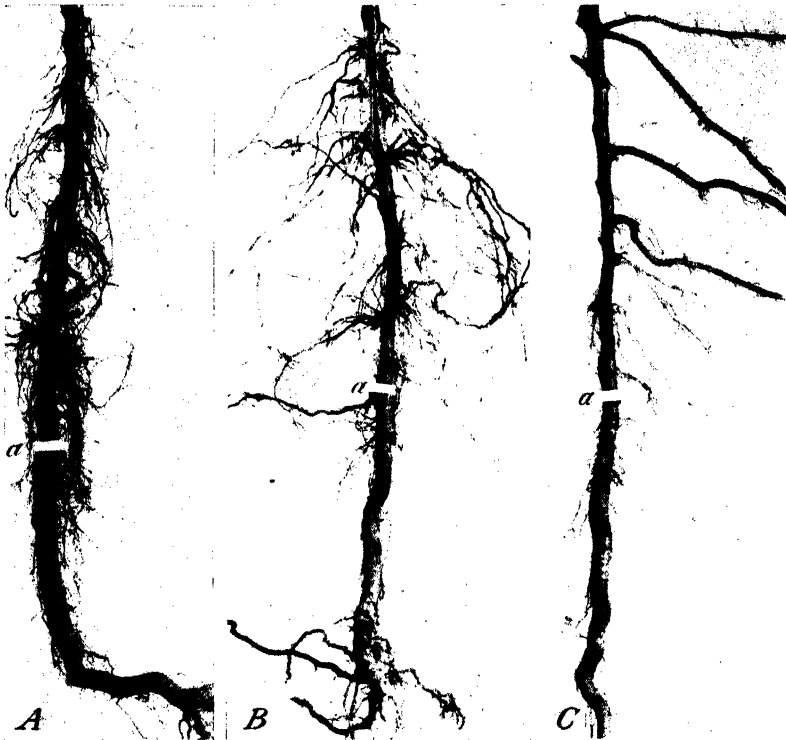


FIGURE 5.—Two-year-old seedlings from 1-year-old layered affected seedlings. Various types of stem rooting are shown, and the symptoms on the original roots have been suppressed. The original root-stem junctions are at *a*. *A* and *B*, More typical examples of the types of stem rooting encountered. *C*, Stem rooting is practically normal in appearance, but there were slight indications of symptoms on some of these roots.

in *C* exhibits what may be tentatively considered normal stem rooting in mode, number, and texture. However, a slight tendency to exhibit noninfectious hairy root symptoms is observed on some of these stem roots. Considerable variation has been observed in the type and number of roots arising on stem tissue, even on the same plant. The 2 plants that did not push stem roots made very poor growth and the symptoms on the original root pieces were pronounced.

DISCUSSION

The reasons for using the term "noninfectious hairy root" have been given. Although the possibility that a virus may be an etiological factor should not be disregarded, there is at the present time no evidence that the condition is infectious. Hedgcock (11) concluded that the condition was not associated with wounding, in contrast with other types of malformations that he described as "soft crown gall,"

"hard crown gall," and "woolly knot" form of hairy root. Muncie's (21) experiments in which he grew apple seed in steamed soil furnished better evidence that the condition is not caused by a pathogen. Other evidence favoring this point of view includes (1) the fact that symptoms have never been observed on the current season's growth of root systems when the older roots are normal; (2) the lack of spread of symptoms to scion part of graft on commercial varieties; (3) the failure of symptoms ever to appear on certain clones that consistently have exhibited normal roots; and (4) the ability either to suppress or to produce the symptoms at will, as demonstrated in these experiments.

The experiments in growing grafts and root cuttings were designed to afford a strict comparison of the reactions of root cuttings with those of grafts, clone for clone. These experiments also furnished a close comparison of the reactions of 1-year-old seedlings with 1-year-old grafts. The regrafting of the stem part of a seedling to its own roots merely simulated the commercial propagation of different varieties by grafting, but it was reasoned that this procedure would afford an opportunity for scion rooting with the elimination of complicating factors due to either mechanical or physiological incompatibilities.

The results of the experiments of 1938 confirm those of previous years and demonstrate that this noninfectious hairy root condition is inherent and that the symptoms are influenced by the degree of stem rooting. The grafts were planted in a normal manner and thus were afforded an opportunity for scion rooting; the root cuttings were planted so as to eliminate rooting from the newly pushed shoots. All of the grafts pushed roots from the stem portion, presumably because of the juvenile condition of the scion wood, and the symptoms of noninfectious hairy root on the original root piece were less marked than they were at the time the grafts were made. However, in the case of the root cuttings the newly pushed shoots did not bear roots and the symptoms were either made manifest or were intensified on the original root piece, depending upon whether it came from a "clean" area or from an area exhibiting symptoms. It follows that the use of any portions of noninfectious hairy root seedlings in propagation will result in manifestations of the symptoms unless the scion variety pushes vigorous scion roots.

The experiments on grafts and on the 1-year-old seedlings are closely comparable. By planting the seedlings horizontally (layering), the stem portions were afforded an opportunity for rooting, and the results are similar to those of the grafting experiment in that stem rooting suppressed the symptoms on the seedling roots.

The experiments with grafts and root cuttings made from normal roots demonstrated that in general the root systems remained characteristic for normal plants. That errors of judgments, however, may be made in classifying seedlings is to be expected, in view of the fact that symptoms are so varied. The fact that over a period of years no symptoms have ever been observed in the thousands of 1-year-old plants grown from root cuttings of U. S. D. A. Vt. 323 is an indication of the constancy of characteristics of root systems, on young plants at least.

For convenience, and in the absence of definite standards for a normal root system, noninfectious hairy root symptoms on 1-year-old seedlings were classified on a quantitative basis as slight, moderate, or pronounced. An excessive number of lateral roots is the chief dis-

tinguishing characteristic, regardless of their morphological arrangement on the main axis. These roots may be either (1) in closely spaced fascicles (burrknot type), (2) in a more or less linear arrangement, or (3) scattered promiscuously around the axis, as illustrated in figure 1, *A*, *B*, and *C*, respectively. Obviously, intergradations occur between all of these arbitrarily selected and somewhat indefinite "types," as well as between normal and affected seedlings. Although these roots generally have a relatively thick phloem region ("cortex"), this more or less "fleshy" condition is apparently usual for abnormally developed roots and presumably is caused by improper functioning. A somewhat distinguishing characteristic is the lack of branching of the smaller roots as compared with normal feeding roots. And, finally, it should be noted that normal-appearing roots may be interspersed along the axis with these abnormal roots.

Two subjects of interest in connection with root systems that display noninfectious hairy root symptoms are (1) the degree of association with aerial burrknots, and (2) the possible dwarfing effect when used as rootstocks. In the absence of evidence obtained in a comparison of clones selected for the purpose of determining these points and propagated by the same methods, a detailed discussion of these questions is not warranted. In general, however, clones that display burrknots are recognized as being good stem rooters (6, 7, 10, 17), and the tufts of roots arising from burrknots may be considered as an abnormal condition similar to noninfectious hairy root (17). It has been observed (10) that although not all Paradise (layered) stocks produce aerial knots, they do develop "tufts of roots resembling those that grow from burr knots."

Unfortunately, there is no experimental evidence on the question as to whether seedlings that exhibit noninfectious hairy root symptoms will, when mature trees, develop root systems similar to those of the dwarfing Malling types of Paradise and other dwarfing rootstocks. The root systems of severely affected 1- and 2-year plants are characterized by regions in which small lateral roots occur in such profusion as to result, presumably, in a restriction of normal functioning. Further, the parts of the root situated distally to (below) regions displaying pronounced symptoms frequently are considerably atrophied and bare of small lateral feeding roots. Thus the root systems of affected plants should eventually become limited in size in the sense that the quantity of lateral roots bearing the finely divided feeding roots will be relatively small.

That a limited size of the root system is an important factor in dwarfing is a general conclusion justified by the evidence submitted by several investigators (12, 25, 26). It is therefore suggested that the responses obtained in the affected plants in these experiments may offer a reasonable explanation for dwarfing on the basis that the number of roots that can function efficiently is relatively small.

These studies indicate that in affected young plants the expression or manifestation of symptoms may be largely dependent upon the influence or dominance of some proximal region. To further illustrate this dominance, and to indicate that the degree of atrophy is greater than is usually encountered, reference is made to Lincoln (15, fig. 1, No. 4) and to Hedcock (11, pl. VII, fig. 1).

That some characteristics may change as the root system becomes older is indicated by descriptions of the dwarfing clone Malling type

IX, which as a 4-year-old tree may exhibit "much fibre chiefly on the upper part of the root system" (34), but later (2) as a 15-year-old tree, on which, however, Bramley's seedling had been grafted, there is no evidence of "fibre," and the long lateral roots bear comparatively few normal finely divided feeding roots.

The above discussion is an attempt to envision the question of dwarfing from a more or less quantitative standpoint. It is desired to emphasize, however, that qualitative factors are undoubtedly of great importance. The anatomical studies on dwarfing stock by Beakbane and Thompson (3) and the studies by Rogers (25) on root growth are of fundamental importance. These workers have given a reasonable explanation for dwarfing, particularly from the standpoint of anatomy (3), and an attempt has been made to establish the idea of root nomenclature as an aid in description (25).

Many factors, such as environment, physiological reactions, age, and nutritional and edaphic conditions, undoubtedly affect the appearance of a root system; but the conclusions of others (24, 34) that most clones have identifying characteristics indicate that the inherent qualities are frequently of decisive importance (1, 2, 6, 12, 33). In order to observe these inherent qualities, it is highly desirable to use methods in propagation and in culture that will result in an optimal expression of growth potentialities in 1- and 2-year-old plants. When these conditions obtain, some differentiation of the factors that may subsequently affect the root systems (1) may be more readily afforded. Tydeman (32) noted the difficulty in classifying "the adventitious systems evolved on" young shoots of stooled seedlings. The present experiments suggest the desirability of using 1- and 2-year-old plants grown from root cuttings as a basis for determining root characteristics of clonal stocks.

The more immediate practical considerations involved in these investigations are concerned mainly with the question of malformations on apple nursery stock. Hedgecock's (11) illustrations and comments on seedlings exhibiting these symptoms of noninfectious hairy root served to warn the writers that this disorder was probably noninfectious, and accordingly his recommendations to discard suspected seedlings for propagation purposes were followed by the writers in experiments on control of crown gall and hairy root. As a result, although in the past nonpathogenic hairy root may have been confused with pathogenic hairy root (19), this disorder has been practically eliminated in all of the writers' experiments since their inception. In the grafting experiments during the last 5 years, however, the writers have noted an occasional noninfectious hairy root tree, particularly in trees grown from domestic seedlings. The results of the present experiments indicate that symptoms may be somewhat masked on a small percentage of 1-year-old seedlings.

In order to eliminate large losses due to culling, seedling growers should give consideration to the fact that the number of seedlings showing this disorder is much larger in the progeny of many of our commercial apple varieties than in that of the French crab. The data in these experiments, supported by observations on general growth habits, however, indicate that seedlings of the Delicious variety are suitable to use (35). Relatively early germination and exceptionally good stands have been obtained from seed of this variety in several

tests. The Delicious variety itself makes exceptionally good root-graft unions, and its seedlings are comparatively uniform.

Although in this discussion emphasis has been placed on the fact that certain rather localized characteristics of a root system can be changed, it should be equally emphasized that the general character of the root system remains constant when conditions favoring vigorous growth are made available. The fact that root systems of vegetatively propagated clones possess distinguishing characteristics is well recognized. Thus, Malling types have been differentiated (5), and Lincoln (14) has illustrated root systems that he considers characteristic of many of our commercial varieties. The latter's illustration of Northern Spy roots appears to show pronounced symptoms of non-infectious hairy root. This variety, in common with the Malling dwarfing clones, is rather readily propagated by layering and is considered a semidwarfing stock (1).

CONCLUSIONS AND SUMMARY

Experiments in which apple seeds from known sources were grown have confirmed field observations that relatively large percentages of 1-year-old seedlings from commercial (domestic) varieties exhibit pronounced symptoms of the disorder formerly known as "simple form hairy root." It is proposed to call this disorder "noninfectious hairy root" in order to emphasize the fact that no pathogenic organism is associated with the condition, which is characterized by an excessive number of lateral roots on the main root axis.

As a result of comparisons of seedlings from various seed sources, including French crab, it is evident that relatively large percentages of seedlings exhibiting these symptoms of this disorder are found in the progeny of many commercial (domestic) varieties. The Delicious variety, however, is an exception, in that a comparatively small percentage of its seedlings are affected.

In order to observe the reaction of affected seedlings under different conditions of growth and to demonstrate the inherent or genetic nature of this disorder, experiments were performed with the use of grafts, root cuttings, and 1-year-old seedlings. Scions from 1-year-old seedlings were grafted to the proximal portions of their own roots; root cuttings were made from the affected portions of these same seedlings and also from regions that appeared normal. In order to preserve the identity of the resulting plants, each original seedling was numbered and treated as clonal material.

The conditions of growth were varied mainly in respect to scion rooting. The use of scion wood from these "juvenile" plants practically insured scion rooting on the grafted plants; by planting the proximal ends of the root cuttings above ground level, scion rooting was precluded.

An examination made 1 year after planting revealed the following conditions:

- (1) On the grafts, scion rooting occurred in all cases, and the symptoms on the original root piece were suppressed, generally in direct proportion to the amount of scion rooting.

- (2) On the 1-year-old seedlings (layered), stem rooting occurred in practically all cases, and the symptoms on the original root pieces were suppressed in agreement with the results obtained on the grafts.

- (3) On the plants grown from root cuttings that had exhibited symptoms when planted, no stem rooting occurred and the symptoms

on the original root piece were intensified. On the plants grown from root cuttings from "clean" (symptomless) regions of affected seedlings, symptoms were manifested.

Because it is the common practice for nurserymen to discard all trees that exhibit symptoms of noninfectious hairy root, it follows that, in order to avoid loss through discarding, no part of affected seedlings should be used for the usual propagation purposes.

Although it is not assumed that all clones that exhibit symptoms will cause dwarfing, it is suggested that selections from clones that exhibit the more pronounced symptoms may readily furnish sources for dwarfing rootstocks.

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THE EFFECT OF CONTROLLED CULLING OF CHICKENS ON THE EFFICIENCY OF PROGENY TESTS¹

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INTRODUCTION

A complete program of progeny testing as visualized today involves compilation and analysis of extensive records on a representative sample of the progeny of the breeding birds under test. There is, however, disagreement as to what constitutes a representative sample. One point of view is that all of the daughters of a given mating hatched during the breeding season should be kept without culling for the whole of the first laying year. A modification of this view, such as practiced under the National Poultry Improvement Plan until the 1939 revision, permits preselection of daughters during the first 60 days of production. A second point of view, brought into practice by many breeders, involves no restrictions with regard to culling. Elimination of unsatisfactory producers under this system may be carried on throughout the year. As a consequence, evaluation of the breeding worth of matings on the basis of only the superior daughters usually results. On the other hand, the advantages of this plan rest in the conduct of breeding work at a reduced cost, due to the saving effected in not having to maintain unprofitable layers and to the possibility of disposal of culls, which would die previous to the end of the year, before their value as market poultry is entirely destroyed.

Recently, Bird and Sinclair³ examined the results from progeny testing with controlled culling, i. e., culling at a constant level for all matings on the basis of egg production records. They suggested that paper culling—disregarding the birds with the lowest annual records in evaluating the results of a mating—may lead to greater accuracy than if the entire flock is used. Furthermore, actual controlled culling throughout the year was suggested as an economy measure.

Bird and Sinclair compared the production means of survivors of three populations described as A, B, and C which were paper-culled at the end of the year. They present differences between B and C at three levels of culling and between populations A and B at two levels. No data are given on results of controlled culling throughout the year. Since only survivors were considered, their data do not indicate how many of the birds which would have been eliminated at any of the periodic cullings under this plan died before the end of the year.

Since the questions raised by Bird and Sinclair are of great importance, it was considered desirable to examine the possibilities of culling practice with respect to progeny testing. Some caution must be exercised in interpreting results such as these, which are based on

¹ Received for publication October 24, 1939.

² The assistance of Burt Wetzel in the preparation of some of the tabular material is acknowledged.

³ BIRD, S., and SINCLAIR, J. W. ON THE VALIDITY OF PROGENY TESTS OF SIRES OBTAINED ON CULLED POPULATIONS OF DAUGHTERS. *Sci. Agr.* 19: 1-6, illus. 1938.

production records. The number of eggs laid by a hen is the phenotypic expression of the action and interaction of many independently transmitted genes, and an analysis of breeding results on the basis of egg production alone leads to a confounding of these component factors. Hence any results obtained by the study of egg records alone are bound to be of limited value.

Furthermore, culling large proportions of a flock may eliminate profitable as well as unprofitable producers. However, the production necessary for a bird to be profitable depends on cost of feedstuffs and price of eggs as well as on the number of eggs laid. No fixed culling percentage can be expected to retain only the profitable birds under varying flock conditions. In this paper several levels of culling are investigated with respect to their effects on evaluation of the progeny test.

MATERIAL AND METHODS

A population of pullets was selected for this study from the University of California flock of Single-Comb White Leghorns. The only standard of selection used was that at least 50 daughters from each sire were to be available. In this manner 12 sire families were obtained in which the number of daughters varied from 52 to 99. The pullets were hatched in 5 weekly hatches in March and April, all pullets alive at 5 months of age being leg-banded and included in this analysis. Differences between the dams mated to the different sires were not considered. This, of course, means that the progeny test as used here applies not to the evaluation of the breeding worth of the 12 sires used, but to that of the mating as a whole, including both the sire and the respective dams mated to each.

Table 1 presents the production and mortality characteristics of the progeny of the 12 sires studied. It may be seen that the 846 pullets involved averaged 151.7 eggs. If the 36.4 percent of the pullets which died are not considered in this computation the average production rises to 199.2 eggs.

TABLE 1.—*Production and mortality of the progeny of the different sires studied*

Sire	Daughters	Mortality of daughters	Production index (eggs)	Production of survivors (eggs)
	Number	Percent	Number	Number
G14	87	25.3	178.2	213.8
G36	79	55.7	93.1	152.3
G52	62	27.4	190.3	225.4
H8	61	36.1	158.0	203.1
H33	52	21.2	162.8	191.2
H42	56	60.7	114.0	193.2
H43	60	38.3	135.4	179.8
H46	68	60.3	111.4	189.5
H62	66	30.3	177.7	226.9
H79	99	24.2	168.7	192.9
H90	95	26.3	165.6	195.8
H91	61	41.0	153.6	203.7
Total	846	36.4	151.7	199.2

The flock was kept intact for the whole year of laying. No culling was actually done, and all of the analyses were carried out after the completion of the year. In this manner it was possible to apply different systems of culling to one and the same population.

The different systems of culling investigated are listed in table 2. System A presupposes no culling whatsoever and the use of the production index (Taylor and Lerner)⁴ obtained by dividing the total number of eggs produced from the first egg to September 30 of the year following the year of hatch by the original number of pullets. Instead of this index the other systems take into consideration only the production of birds which survived to October 1 of their second year of life. System B again involves no culling, while systems C, D, E, F, and G designate respectively the culling of the 12, 18, 24, 30, and 36 percent of each sire family which are lowest in egg production. The subscripts indicate the frequency of culling during the year. Thus the subscript 1 denotes paper culling on October 1, at the end of the laying year under consideration. Subscripts 3, 4, and 6 indicate the given number of cullings throughout the year. As shown in table 2, on the first of each of the particular months designated, the lowest 2, 3, 4, 4.5, 6, and 8 percent (depending on the total level of culling in each system) of the original number of pullets in each sire family were considered as having been culled. Only birds alive on each particular date were thus treated, the culling being in addition to mortality. All of the culling was based on the production up to the particular date on which the culling was presumed to have taken place.

TABLE 2.—*Description of culling systems*

System	Proportion culled	Culling procedure	Birds remaining after final culling
	Percent		Number
A	0	All birds banded considered	846
B	0	Only survivors considered	538
C ₁	12	Paper-culled in October	439
D ₁	18	do	386
E ₁	24	do	335
F ₁	30	do	283
G ₁	36	do	234
C ₃	12	3 cullings of 4 percent each (February, June, October)	475
D ₃	18	3 cullings of 6 percent each (February, June, October)	438
E ₃	24	3 cullings of 8 percent each (February, June, October)	402
C ₄	12	4 cullings of 3 percent each (January, April, July, October)	482
D ₄	18	4 cullings of 4.5 percent each (January, April, July, October)	451
E ₄	24	4 cullings of 6 percent each (January, April, July, October)	412
C ₆	12	6 cullings of 2 percent each (December, February, April, June, August, October).	486
D ₆	18	6 cullings of 3 percent each (December, February, April, June, August, October).	461
E ₆	24	6 cullings of 4 percent each (December, February, April, June, August, October).	432

It should be clearly understood that each sire family was culled independently of any other family, so that the level of culling in a given system was the same for each sire family. Systems F₁ and G₁ were not complemented with systems of continual culling at the same total level, because, in the case of some families at least, such heavy culling plus the mortality that occurred would have reduced the families to the vanishing point. The total number of daughters involved under each system is also given in table 2. It may be noticed that some discrepancies in number of birds appear between systems in which the total amount of culling was the same. The reason for this, of course, lies in the fact that some of the birds culled on the

⁴ TAYLOR, LEWIS W., and LERNER, I. MICHAEL. BREEDING FOR EGG PRODUCTION. Calif. Agr. Expt. Sta. Bul. 626, 48 pp., illus. 1938.

basis of their low production early in the year died before they were eligible for culling under another system.

The analyses of the above systems were designed to test whether culling fulfills either of its two possible purposes in breeding flocks: (1) To increase the precision of progeny testing by increasing the significance of differences between means of sire families while preserving the relative order of ranking, and (2) to effect managerial economies.

The fulfillment of the first of these purposes may be examined by comparing the variability between and within sire families under the different culling systems. The ratio of the variance between to that within sire families provides a convenient criterion. It is, however, necessary to supplement this measure with a check on the rank order of these sire families. The fulfillment of the second purpose may be examined by the actual comparison of hen-months involved under each system, together with the consideration of the salvage value of the culls, which would have died before the expiration of the year.

The analyses made follow this outline. The only difficulty in interpreting the results lies in the necessity of assuming a normal distribution in each of the sire families, without which a precise study of the variance is not possible. While normal distributions in general are not the rule, this assumption may be justified to a certain extent by the following considerations. Whether or not culling is practiced, the determination of the superiority or inferiority of any given mating has to be made by a comparison of the mean production of this mating with other matings. Truncation (elimination of a part of the curve) and reconstruction of a normal curve from the truncated distribution will obviously yield a value different from the observed mean production. Assumption of normality introduces a decided error in variance analysis but does not lend greater bias to any mating over any other than does the truncation. It is, perhaps, possible to normalize the frequency curves by the choice of suitable coordinates, but in any case these coordinates may be variable from mating to mating. Furthermore, the labor involved and the statistical training necessary for this would preclude any possibility of using such methods in practical breeding work.

RESULTS

PRECISION OF PROGENY TESTS

The mean production of the flock, the variances between and within sire families, and the ratio of these variances for each of the culling systems investigated are shown in table 3. It may be seen that system A exhibits the largest variance both between and within sire families, the ratio of the mean squares also being the largest. Under this system, it will be recalled, mortality and production are evaluated together, so that the net efficiency of the different matings is compared. When production of survivors only is considered, as in all of the other systems, both the variance between and the variance within sire families show a precipitous drop. However, when paper culling only is considered (systems with subscripts of 1), the former drops to a greater extent than the latter. This is shown by the reduction in the ratio of mean squares as compared to the ratio observed under system A. So far as comparison with system B (no culling) is concerned, there is little difference in the ratio of mean squares in the

single culling systems, except for F_1 and G_1 which show a considerably higher variance ratio. They are still greatly below the ratio obtained with system A. More frequent culling increases this ratio over that obtained with system B, but in no case is it as high as the ratio of 11.56 of system A.

TABLE 3.—*Statistical constants of different culling systems*

(1) System	(2) Mean production	(3) Mean square between sires	(4) Mean square within sires	(5) Ratio of mean squares (column 3: column 4)	(6) Number of significant differences between sires
A.....	151.7	79,571	6,883	11.56	32
B.....	199.2	16,506	3,286	5.02	24
C ₁	221.2	8,608	1,579	5.49	32
D ₁	228.8	8,829	1,624	5.44	27
E ₁	235.8	7,311	1,810	4.04	20
F ₁	239.5	5,689	647	8.79	28
G ₁	245.0	4,695	591	7.94	26
C ₂	212.9	13,442	1,743	7.71	34
D ₂	218.7	12,169	1,392	8.74	34
E ₂	224.2	9,667	1,131	8.55	33
C ₃	210.5	16,388	1,070	8.28	32
D ₃	215.5	15,575	1,658	9.39	35
E ₃	222.1	11,144	1,254	8.89	35
C ₄	206.6	17,778	2,502	7.11	28
D ₄	211.0	20,131	2,072	9.72	36
E ₄	215.9	18,322	1,737	10.55	37

Perhaps a more pertinent manner of comparison is to calculate the number of significant differences between the various sire families in each system, the determination of such significance to be made on the basis of the standard deviations and number of degrees of freedom available from each mating. With 12 sire families, if every family is significantly different from every other, 66 significant differences would be present. The last column of table 3 shows the number of such differences found, based on the actual degrees of freedom available in each mating under each system. They range from 20 for system E_1 to 37 for E_6 . It is apparent that single culling on the whole shows the lowest number of significant differences, while more frequent cullings, on the other hand, tend to show a greater number of such differences.

TABLE 4.—*Comparative amounts of useful differentiation available in each culling system*

System	Significant differences as percent of total possible differences	Differentiation when quartiles are based on all birds			Differentiation when quartiles are based on survivors		
		High quartile	Low quartile	High +low	High quartile	Low quartile	High +low
	Percent	Percent	Percent	Percent	Percent	Percent	Percent
A.....	48.5	42.4	75.8	59.1	42.4	69.7	56.1
B.....	36.4	45.5	54.5	50.0	45.5	57.6	51.6
C ₁	48.5	54.5	42.4	48.5	54.5	54.5	54.5
D ₁	40.9	45.5	27.3	36.4	45.5	45.5	45.5
E ₁	30.3	42.4	12.1	27.3	42.4	36.4	39.4
F ₁	42.4	60.6	15.2	37.9	60.6	33.3	47.0
G ₁	39.4	57.6	12.1	34.9	57.6	30.3	44.0
C ₂	51.5	57.6	51.5	54.6	57.6	57.6	57.6
D ₂	51.5	63.6	51.5	57.6	63.6	57.6	60.6
E ₂	50.0	60.6	42.4	51.5	60.6	48.5	54.6
C ₃	48.5	57.6	48.5	53.1	57.6	60.6	59.1
D ₃	53.0	60.6	54.5	57.6	60.6	66.7	63.7
E ₃	53.0	63.6	51.5	57.6	63.6	60.6	62.1
C ₄	42.4	54.5	45.5	50.0	54.5	57.6	56.1
D ₄	54.5	72.7	57.6	65.2	72.7	63.6	68.2
E ₄	56.1	63.6	57.6	60.6	63.6	69.7	66.7

The nature of the differences observed is analyzed in table 4. The second column shows the numbers listed in the last column of table 3 as percent of the total possible differences (66). These percentages can be broken down by consideration of the section of the distribution of the means of sire families in which the significant differences occur. The particular utility of differentiation between sire families lies in the upper limits of the distribution (selection of superior matings) and in the lower limits (elimination of inferior matings), rather than in the midportion of the distribution. Hence, the differentiation of the upper and lower quartiles from the other families may be designated as useful differentiation.

While the different systems may be compared among themselves, it is desirable to designate the quartile order of sire families in one system as a standard in order to be able to pass judgment with regard to such differentiation. System A, based on the production index, measures the actual efficiency of production, taking both egg production and mortality into consideration. Hence it was used as a base. For purposes of distinguishing the factors determining egg production from those affecting mortality, system B, involving only survivors, was chosen as another base.

The percentages of total possible useful differentiation were thus calculated for each of the systems on two bases, that of ranking with regard to the production index (all birds) and to the production of survivors. These percentages are shown in table 4. Once more multiple cullings show an increase in percentage of differentiation over that observed in single cullings. However, the differences between the culled and unculted groups are found to be largely in the differentiation of the upper quartile of sire families. In the lower quartile, system A shows a considerably higher percentage of differentiation. In other words, culling as here investigated makes it easier to select out the better matings, but makes it harder to distinguish the inferior matings.

The next step involves the question of preservation of the rank order of the different sire families in each of the culling systems. As in table 4, systems A and B served as the two bases. Table 5 presents

TABLE 5.—*Coefficients of rank-order correlation between sire families in each system*

System	Correlated with system A		Correlated with system B		System	Correlated with system A		Correlated with system B	
	ρ	ρ^2	ρ	ρ^2		ρ	ρ^2	ρ	ρ^2
B.....	0.779	0.61	1.000	1.00	E ₁476	.23	.782	.61
C ₁538	.29	.860	.74	C ₁573	.33	.845	.71
D ₁441	.19	.762	.58	D ₁552	.30	.831	.69
E ₁378	.14	.734	.54	E ₁476	.23	.775	.60
F ₁291	.08	.650	.42	C ₂577	.33	.870	.76
G ₁238	.06	.622	.39	D ₂650	.42	.851	.72
C ₂497	.25	.796	.63	E ₂497	.25	.796	.63
D ₂448	.20	.747	.56					

coefficients of rank-order correlation of sire families between each of the systems and the two bases, namely, all birds (A) and survivors (B). It will be seen that the correlation coefficients tend to drop with increased culling. Less than 45-percent determination (ρ^2) is available, when comparison of the systems involving culling is made with system A, system G₁ falling as low as 6 percent, while system B shows

over 60-percent determination. The coefficients for the culled groups are considerably higher when comparison with system B is made.

It may thus be seen that a high ratio between mean squares, such as observed in system E₆ (table 3) is not in itself a guarantee of increased precision of differentiation between sires, since in this particular instance the rank order of sire families bears only little relation to that in the unculled population (table 5). Conversely, a low ratio, such as is found in system B may still be associated with a high coefficient of rank-order correlation.

ECONOMIES DUE TO CULLING

The systems that involve culling at the end of the year do not effect any economies. On the other hand, systems of culling three, four, and six times a year produce twofold economies: (1) The salvage meat value of the culled birds, which otherwise would have died in the course of the year, and (2) the saving on feeding of the culled birds. Table 6 presents estimates of such economies for this flock. It may

TABLE 6.—*Economy effected under each system of culling*

System	Number of birds	Culls salvaged as proportion of -		Saving on feeding	
		All dead birds of system A	All culls of each system	Hen-months	Percent of all hen-months
		<i>Percent</i>	<i>Percent</i>	<i>Number</i>	<i>Percent</i>
C ₃	35	11.36	35.35	360	3.55
D ₃	51	16.56	33.55	467	4.60
E ₃	67	21.75	33.00	628	6.19
C ₄	43	13.96	43.44	407	4.01
D ₄	66	21.43	43.42	609	6.00
E ₄	76	24.68	37.44	759	7.48
C ₆	47	15.26	47.47	695	6.85
D ₆	76	24.68	50.00	937	9.23
E ₆	97	31.49	47.78	1,118	11.01

be seen that under the 12-percent culling system, 11 to 15 percent of the birds dying annually in the unculled flock can be converted into market hens. Under the 18-percent culling system, these figures rise to 16 to 25 percent; and with 24-percent culling, from 21 to 31 percent of the birds which would die before the end of the first year of production can be sold for meat. This represents from 33 to 50 percent of all of the culled birds, according to the amount and frequency of culling.

The saving in feed and other expenses coincident with the keeping of the unprofitable producers for the whole year varies from 3 to 11 percent of the total hen-months, again according to the amount and frequency of culling.

PRECISION OF LIVABILITY TESTS

Table 7 presents the correlation coefficients of mortality rank order under the various culling systems. Again, paper culling at the end of the year does not affect these ranks. It may be seen that the correlations obtained between the nonculled and culled sire families are all above 0.92, when total mortality is considered, and above 0.93 for mortality of neoplastic origin. This suggests that selection against

high mortality can be carried on with practically the same degree of efficiency under the systems of culling investigated here as on a non-culled population.

DISCUSSION AND CONCLUSIONS

The work of Bird and Sinclair ⁵ seems to be the only investigation to date concerning the effect of culling on the progeny test. As already mentioned, these workers examined the records of survivors of three populations of pullets of unspecified origin. The data presented here differ from their material both in scope and in the methods of analysis. Here, comparisons are made of 12 populations of pullets originating from 12 different sires. Mortality was taken into account both in relation to the precision of the progeny test and in relation to managemental economies. The birds whose records were studied were hatched in a period of 4 weeks and carried through the first laying year in a manner closely approaching that used in practical breeding work. The application of the statistical methods employed has been discussed previously in the presentation of results.

TABLE 7.—*Coefficients of rank-order correlation with respect to mortality under different culling systems*

System	Correlation with actual mortality		System	Correlation with actual mortality	
	Total	Neoplastic		Total	Neoplastic
C ₃	0.958	0.965	E ₄	0.972	0.965
D ₃923	.965	C ₅972	.970
E ₃923	.965	D ₄972	.972
C ₄951	.972	E ₅965	.937
D ₄965	.965			

Tables 4 and 5 may be considered to summarize the findings with regard to the comparative efficiency of segregation of superior and inferior sire families under systems with and without culling. It is apparent that the total percentage of useful differentiation based on all birds is not increased in the single culling systems over that of system A. Furthermore, the coefficients of rank-order correlation are of comparatively low magnitude both for single and multiple culling systems. Systems of culling throughout the year yield much higher percentages of useful determination in the upper quartile of the distribution of egg production means, but fall below that of system A in the lower quartile. At best they equal system B. Only systems D₆ and E₆ exceed the total percentage of useful determination obtained under system A. However, even with these it may be seen from table 3 that the ratio of mean squares between to that within families is still the highest under system A. So far as the coefficients of rank-order correlation with respect to mortality are concerned, very high values are obtained in all of the systems considered.

The economies as observed in the flock exceed 10 percent of feeding costs only in one case (that of system E₆), while up to approximately 30 percent of the birds dying annually can be salvaged for market-meat purposes under systems of culling throughout the year.

⁵ See footnote 3.

In drawing conclusions from the data here presented, a possible adverse effect of culling practices on the measurement of individual inherited characters, which are components of the annual egg record, has not been considered. Before any degree of culling of breeding flocks may be advocated without qualification, this possibility should be eliminated.

The ultimate answers to the questions raised in this paper may be found only in actual breeding tests of birds selected under the different systems. However, from the data here presented it may be concluded that, when annual egg production and livability are the gross desiderata considered:

- (1) Paper culling at the end of the year is of questionable value.
- (2) No system of culling investigated in this paper gives greater differentiation between matings than the system used in the calculation of the production index, which involves both production and mortality characters.
- (3) Controlled culling throughout the year at levels of 12 to 24 percent enhances the possibilities of selecting the sire families adjudged superior by the production index, at the same time decreasing precision in the elimination of inferior families.
- (4) Such systems of culling do not affect to any great extent the accuracy of determination of the rank order of sire families with respect to first-laying-year mortality.
- (5) Economies effected by culling in the flock include 3 to 11 percent of the feeding cost and the conversion into market meat of 11 to 31 percent of the birds which would die during the first laying year.

ACTION OF SOME ORGANIC COMPOUNDS ON YIELD, SPORULATION, AND STARCH FORMATION OF *ASPERGILLUS NIGER*¹

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INTRODUCTION

Studies dealing with the action of organic compounds on the metabolism of organisms have assumed considerable importance, and much activity now exists in the fields concerned with vitamins, hormones, therapeutic compounds, insecticides, fungicides, and compounds capable of inducing carcinomas. Relatively few such investigations, however, have concerned themselves with *Aspergillus niger* Van Tiegh., though it is one of the two fungi whose nutrition is best understood.

The compounds selected for study were therefore chosen for different reasons. The effects of the 37 biological stains are of interest because of their use as "vital" stains, their toxicity, and their property of fluorescence for which claims have appeared in the literature (7).² The 85 phenanthrene derivatives were included for somewhat similar reasons and particularly because of their similarity in chemical structure to morphine and certain animal hormones. Miscellaneous compounds, to the number of 21, included alkaloids, pharmaceutical preparations, cancer-producing derivatives, and a few dyes of complicated structure. The effects of these 143 compounds have been observed on yield, sporulation, formation of starch, and ability to produce mutants. The genetical studies, however, have been reported elsewhere (10).

The data throw additional light on the subject of "chemical stimulation" of plants (9) and the antagonistic relation claimed to exist between growth and reproduction. Observations dealing with the effects of low sucrose, low nitrogen, or low trace elements on the action of the substances studied should also prove of interest in this connection.

Little is known concerning the conditions requisite for the formation of starch from sugar by fungi. Lappalainen (5) considered starch a normal constituent formed under good nutritional conditions. According to Boas (1), however, starch is a pathological product resulting from the destruction of diastase under the influence of high acidity. The presence of ammonium salts was considered to favor the formation of starch because of its physiological acidity. Starch was found by Boas to be formed from sugars, higher alcohols, and organic acids. Schmidt (6) found that nitrates inhibit starch formation. In the opinion of Chrzaszcz and Tiukow (2), the elaboration of starch by fungi is characteristic of low-acid producers. Forty-five species were studied.

¹ Received for publication April 1, 1940.

² Italic numbers in parentheses refer to Literature Cited, p. 773.

EXPERIMENTAL METHODS

Cultures of *Aspergillus niger* (No. 4247W in the collection of Dr. Charles Thom, of the Bureau of Plant Industry) were grown for 4 days at 35° C. in 200-cc. pyrex Erlenmeyer flasks containing 50 cc. of nutrient solution. The composition per liter of the basic nutrient solution was as follows: Sucrose, 50 gm.; ammonium nitrate, 2.00 gm.; dipotassium phosphate, 0.35 gm.; and magnesium sulfate (7H₂O), 0.25 gm.; iron, zinc, copper, manganese, and molybdenum, 0.30, 0.20, 0.05, 0.03, and 0.02 mg. per liter, respectively. Each constituent in this solution was adjusted for maximum yield. Reagent chemicals were used in preparing the solution. The water was redistilled in a pyrex glass still. The sucrose had an ash content of about 0.002 percent. Flasks were sterilized in the steamer for 20 minutes, and inoculation was effected with a spore suspension.

The cultures were filtered, when harvested, with fritted glass crucibles of No. 3 porosity. To indicate the presence of starch, a drop of iodine (N/20) was placed on the reverse of the mycelial felts before they were washed on the filter. It was necessary to wait an hour or more before deciding on the presence or absence of starch. Examination at a 10× magnification was usually advisable. The crucibles containing the mycelial felts were then placed in a current of warm air (45° C.) for 3 to 4 hours, and finally in the oven at 103° overnight. Filtrates from the mycelial felts were also tested for the presence of starch. The quantities of iodine necessary per culture varied considerably under different conditions. No record was made of these variations despite the possibility that ascorbic acid might be concerned therein.

The organic compounds³ employed in these experiments were each examined under a 5-ampere, black-bulb, mercury-vapor lamp to determine whether they exhibited fluorescence.

EXPERIMENTAL RESULTS

EFFECTS OF BIOLOGICAL STAINS

In table 1 are summarized the results obtained with a number of biological stains when present in the cultures in a concentration of 10 parts per million. The first series was with low sucrose. In no case did addition lead to an increase in growth above that of the control greater than 4.6 percent. The minimum significant variation is considered to be 5 percent. Sporulation generally paralleled growth, but not invariably. Alizarine red S gave a yield of 71.4 percent of maximum, whereas sporulation was depressed to about 12 percent of maximum. Rhodamine B gave identical results on yield and sporulation. The difference in fluorescence between these compounds appeared to be without effect. No general relation, moreover, appears to exist between toxicity, fluorescence, sporulation, or starch formation.

A 50-percent decrease in ammonium nitrate was accompanied in many instances by a diminution in toxicity of these dyes, both with respect to growth and reproduction. The maximum increase in

³ The phenanthrene derivatives were obtained through the courtesy of Dr. Erich Mosettig, of the U. S. Public Health Service; Federal Security Agency; and the acridine, indanthrone, and indigo derivatives (see table 2), from Dr. W. H. Tisdale, of the Pest Control Research Section, E. I. du Pont de Nemours & Co., Inc.

yield above that of the control was 4 percent. Sporulation usually diminished with decrease in growth, as did also starch. Eosin, erythrosin, and Martius yellow gave cultures with enhanced starch content. Partial nitrogen deficiency in this series had an adverse effect on starch formation.

TABLE 1.—*Effect of some biological stains on growth, sporulation, and starch formation by Aspergillus niger at 35° C. for 4 days in a dibasic optimum solution*¹

Compound ² (10 mg. per liter)	Low sucrose (80 percent of optimum)				Low nitrogen (50 percent of optimum)				Low trace elements (50 percent of optimum)			
	Yield ³	Sporulation ⁴	Starch in ⁵ —		Yield ³	Sporulation ⁴	Starch in ⁵ —		Yield ³	Sporulation ⁴	Starch in ⁵ —	
			Mycelium	Solution			Mycelium	Solution			Mycelium	Solution
Control.....	Percent 100.0	8, bl	3	0	Percent 100.0	10, bl	3	0	Percent 100.0	4, bl	5	5
*Acridine orange.....	34.5	0	5	5	47.3	0	0	0	55.3	0	5	5
*Acridine red.....	93.8	3, bl	3	0	87.0	2, bl	2	0	56.8	0	5	5
*Acridine yellow.....	40.6	0	5	5	69.1	4, bl	0	0	67.7	2, bl	5	5
*Acridiflavine (neutral).....	97.0	8, bl	3	0	97.1	10, bl	0	0	98.3	2, bl	5	5
Alizarine blue (Ehrlich).....	50.7	2, bl	5	5	93.1	2, y	2	2	56.5	2, bl	5	5
Alizarine red S.....	71.4	1, bl	2	0	93.5	4, bl	1	0	73.7	0	5	5
Aniline Blue.....	102.2	8, bl	0	0	97.2	10, bl	3	0	111.4	2, bl	5	5
Auramine O.....	100.4	8, bl	1	0	100.5	10, bl	2	0	104.4	4, bl	5	5
Brilliant alizarine.....	103.2	8, bl	0	0	98.7	10, bl	3	0	119.4	4, bl	5	5
Brilliant indigo.....	91.7	4, bl	5	0	91.2	8, bl	2	0	99.6	4, bl	5	5
Chrysoidine.....	99.1	4, bl	3	0	98.0	10, bl	2	0	94.3	2, bl	5	5
*Eosin, W gelb.....	101.1	2, bl	5	0	104.0	8, bl	5	0	112.9	0	5	(e)
*Erythrosin bluish.....	104.6	8, bl	5	0	94.6	10, bl	5	0	114.2	2, bl	5	(e)
Ethyl red.....	100.5	8, bl	7	0	100.3	10, bl	3	0	120.3	4, bl	5	5
*Fluorescein.....	99.7	8, bl	7	0	98.7	10, bl	2	9	106.6	3, bl	5	5
Fuchsin S.....	98.8	6, bl	5	0	97.9	10, bl	3	0	116.6	3, bl	5	5
*Indigo carmine.....	102.1	6, bl	5	0	93.0	8, bl	1	0	100.0	2, bl	5	5
Malachite green (crystalline).....	0	0	0	0	0	0	0	0	0	0	0	0
Martius yellow.....	86.5	2, bl	3	0	64.7	2, bl	5	0	84.8	0	3	(e)
*Meldola blue.....	67.3	1, bl	5	5	82.7	4, bl	2	2	62.0	0	5	5
*Methyl violet.....	0	0	0	0	0	0	0	0	0	0	0	0
Methylene blue.....	57.1	1, bl	5	5	73.8	1, bl	1	0	70.3	1, bl	5	5
Methylene violet.....	86.4	6, bl	5	0	90.4	8, bl	3	0	83.4	3, bl	5	5
Naphthol green.....	95.2	4, bl	0	0	92.7	8, bl	2	0	121.2	2, bl	5	5
Nile blue sulfate.....	0	0	0	0	0	0	0	0	0	0	0	0
Orange G.....	99.8	4, bl	7	0	97.8	10, bl	3	0	154.4	2, bl	5	5
Phosphine.....	102.1	4, bl	5	0	103.3	8, bl	2	0	131.0	2, bl	5	2
*Proflavine.....	97.9	8, bl	3	9	100.0	10, bl	2	0	100.4	2, bl	5	5
Quinoline yellow.....	104.5	8, bl	7	0	101.3	10, bl	3	0	134.2	3, bl	5	5
*Rhodamine B.....	71.4	1, bl	5	7	85.6	2, bl	1	0	84.0	2, bl	5	5
Rhoduline GO.....	48.1	1, bl	5	7	84.6	6, bl	1	0	49.1	0	5	3
Rose Bengal.....	93.0	4, bl	5	0	92.1	6, bl	3	0	113.2	2, bl	5	3
Safranin Ow.....	85.9	4, bl	5	0	99.3	8, bl	3	0	70.2	3, bl	5	5
Tartrazine.....	102.2	8, bl	0	0	98.6	10, bl	3	0	141.5	2, bl	5	5
Thionine.....	100.8	8, bl	2	0	101.8	10, bl	2	0	148.0	2, bl	5	(e)
Trypan blue.....	102.1	8, bl	2	0	97.0	10, bl	3	0	104.8	4, bl	5	5
Trypan red.....	95.7	6, bl	3	0	82.4	6, bl	0	0	63.0	3, bl	5	5

¹ Nutrient solution: Water, 1,000 cc.; sucrose, 50 gm.; NH_4NO_3 , 2.06 gm.; K_2HPO_4 , 0.35 gm.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.25 gm.; and Fe, Zn, Cu, Mn, and Mo, 0.20, 0.20, 0.05, 0.03, and 0.02 mg. per liter, respectively.

² An asterisk * before the name of a compound denotes that it was found to fluoresce in ultraviolet light.

³ Yield of control culture taken as 100 percent.

⁴ Sporulation is indicated on a scale of 0 (sterile) to 10 (black with spores), and spore color by the initial letters of the words black, brown, and yellow.

⁵ Starch is indicated on a scale of 0 (none) to 5 (abundant). Tests giving an immediate blue color are indicated by italics.

⁶ Erythrodxtrin indicated by red color.

The results obtained with an insufficient quantity of trace elements for maximum yield gave perhaps the most interesting results. Many instances of yields greater than that of the control were obtained, ranging up to an increase in maximum yield of 54.4 percent with

orange G. Examples are also to be found of the so-called "stimulation" response, namely, decreased sporulation accompanied by increased growth. Starch formation was abundant with trace-element deficiency, and to such an extent that the filtrates of almost every culture gave a very strong test for starch.

Cultures with 10 mg. per liter of malachite green, methyl violet, or Nile blue sulfate did not germinate within the duration of the experiments. Further tests with 1 mg. per liter gave practically maximum yields with the two last-named compounds though sporulation was decreased by half, and Nile blue sulfate appeared to increase starch formation slightly. The cultures with 1 mg. of malachite green per liter again did not germinate. Guilliermond and Gautheret (4) found malachite green quite toxic to roots of wheat seedlings.

EFFECTS OF MISCELLANEOUS ORGANIC COMPOUNDS

The effects of miscellaneous organic compounds (table 2) are quite similar on the whole to those obtained with biological stains (table 1). Comparison of the three series will reveal that here also low nitrogen increased reproduction and decreased the formation of starch, whereas low trace elements resulted in diminished sporulation and a marked increase in starch production. Moreover, only with low trace elements did increases in growth above that of the control occur upon addition of organic compounds.

TABLE 2.—Effect of miscellaneous organic compounds on growth, sporulation, and starch formation by *Aspergillus niger* at 35° C. for 4 days in a dibasic optimum solution¹

Compound (10 mg. per liter)	Low sucrose (80 percent of optimum)				Low nitrogen (50 percent of optimum)				Low trace elements (50 percent of optimum)			
	Yield	Sporulation	Starch in—		Yield	Sporulation	Starch in—		Yield	Sporulation	Starch in—	
			Myce- lium	Solu- tion			Myce- lium	Solu- tion			Myce- lium	Solu- tion
	Percent				Percent				Percent			
Control	100.0	8, bl	2	0	100.0	10, br	3	0	100.0	4, bl	5	5
Aceonaphthene	97.9	8, bl	3	0	100.2	10, br	3	0	101.7	4, bl	5	5
Acridine	99.6	8, bl	3	0	99.0	10, br	3	0	106.0	4, bl	5	5
Barbituric acid	99.6	8, bl	5	0	98.9	10, br	3	0	94.7	4, bl	5	5
Benzidine sulfate	97.8	8, bl	3	0	97.6	10, br	3	0	133.0	4, bl	5	5
Caffeine	101.1	8, bl	3	0	92.3	6, br	2	0	80.6	4, bl	5	5
Colchicine	102.1	8, bl	3	0	95.6	8, br	3	0	92.3	4, bl	5	5
*1, 2, 5, 6-Dibenzanthracene	100.2	8, bl	3	0	96.7	8, br	3	0	92.4	4, bl	5	5
Dibrom indanthrone	96.3	8, bl	2	0	98.1	10, br	3	0	116.5	4, bl	5	0
*Dibrom indigo	95.6	6, bl	2	0	94.2	8, br	3	0	112.9	4, bl	5	5
Dichlor indanthrone	98.4	8, bl	2	0	97.4	8, br	3	0	108.6	4, bl	5	5
Digitonin	86.8	4, bl	2	0	81.0	4, br	3	0	80.9	4, bl	5	5
Flavanthrone	96.6	8, bl	1	0	96.2	8, br	3	0	124.3	4, bl	5	5
Indanthrone	97.0	8, bl	2	0	99.4	10, br	3	0	134.5	4, bl	5	5
*Methylcholanthrene	98.9	8, bl	2	0	98.2	10, br	3	0	102.7	4, bl	5	5
*Dibrom indanthrone	97.5	8, bl	2	0	98.0	8, br	3	0	111.7	4, bl	5	5
Phenanthrene	98.0	6, bl	2	0	95.5	6, br	3	0	108.0	4, bl	5	5
*Quinine hydrochloride	100.8	8, bl	3	0	98.7	10, br	3	0	103.3	3, bl	5	5
Saponin	88.3	2, bl	2	0	88.0	2, br	2	2	91.2	4, bl	5	5
Sulfanilamide	97.8	8, bl	2	0	99.3	10, br	3	0	78.6	4, bl	5	5
Tetrabrom indigo	92.4	8, bl	3	0	90.3	4, br	2	0	101.9	4, bl	5	5
Theobromine	97.3	7, bl	3	0	102.0	10, br	3	0	113.0	2, bl	5	5

¹ Footnotes 1 to 5 of table 1 apply also to this table.

EFFECTS OF PHENANTHRENE DERIVATIVES

In the experiments of table 3 the cultures were grown on a low-sucrose solution containing 100 p. p. m. of the different phenanthrene derivatives. It will be noted that the property of fluorescence of the various compounds bore no relation to any of the results obtained. The maximum increase in yield over that of the control amounted to 8.9 percent with compound St 53. Compounds St 8, St 11, St 15, and St 25 also gave increases in weight of over 5 percent. Only derivatives St 14 and St 15 gave increased sporulation; the increase, however, was but slight. The most marked of the increases in starch formation was brought about by St 6 and St 44. Greatly increased starch formation also resulted from addition of compounds St 1, St 14, St 21, St 33, St 36, St 39, St 40, St 41, St 43, St 46, St 48, St 53, St 57, St 58, St 61, St 76, and St 77. Many of the compounds causing increased production of starch were carboxylic acids or hydrochloride salts.

TABLE 3.—*Effect of some phenanthrene derivatives (at 100 mg. per liter) on growth, sporulation, and starch formation by Aspergillus niger at 35° C. for 4 days in a dibasic optimum solution*¹

Compound No.	Phenanthrene derivative (100 mg. per liter, or 0.01 percent)	Yield	Sporulation	Starch in—	
				Mycelium	Solution
		<i>Percent</i>			
	Control	100.0	8, b1	2	0
St 1	1-oxo-1, 2, 3, 4-tetrahydrophenanthrene	69.0	1, b1	5	0
St 2	4-oxo-1, 2, 3, 4-tetrahydrophenanthrene	0	0	0	0
St 3	3-acetoxy-6-acetylphenanthrene	85.6	4, b1	3	0
*St 4	Phenanthrene-3-aldehyde	83.5	2, b1	4	0
St 5	Phenanthrene-9-aldehyde	93.3	6, b1	4	0
St 6	9-hydroxyphenanthrene-10-aldehyde	52.5	1, b1	5	3
St 7	3-methoxy-phenanthrene-9-carboxylic acid	88.5	4, b1	3	0
St 8	2-propionylphenanthrene	107.0	8, b1	2	0
St 9	2-hydroxy-9, 10-dihydrophenanthrene	0	0	0	0
St 10	2-hydroxy-3, 7-dipropionyl-9, 10-dihydrophenanthrene	101.5	8, b1	3	0
St 11	2-hydroxy-3, 7-di-n-butyl-9, 10-dihydrophenanthrene	106.4	8, b1	2	0
St 12	Phenanthrene	97.5	4, b1	2	0
St 13	9, 10-phenanthrenequinone	3.5	2, b1	0	0
St 14	2-(1-hydroxy-n-propyl)phenanthrene	96.3	10, b1	5	0
*St 15	1-hydroxy-2-acetylphenanthrene	105.7	10, b1	4	0
*St 16	4-hydroxydiacetylphenanthrene	99.7	2, b1	2	0
*St 17	4-hydroxydipropionylphenanthrene	102.7	4, b1	4	0
St 18	3-(2-isonitroso-1-oxo-propyl)phenanthrene	103.8	4, b1	2	0
St 19	3-(2-amino-1-hydroxy-n-propyl)-phenanthrene hydrochloride	0	0	0	0
*St 20	3-(2-piperidino-1-hydroxy-n-propyl)phenanthrene hydrochloride	0	0	0	0
St 21	9-(2-amino-1-oxo-propyl)phenanthrene hydrochloride	79.7	2, b1	5	0
St 22	Phenanthrene-2-carboxylic acid methyl ester	101.2	4, b1	4	0
St 23	Phenanthrene-2-carboxylic acid ethyl ester	90.4	4, b1	2	0
St 24	3-phenanthryl-methanol	59.0	4, b1	5	0
St 25	9-phenanthryl-methanol	106.1	8, b1	3	0
St 26	9-ethyl-phenanthrene	101.6	8, b1	3	0
St 27	2-(1-hydroxyethyl) phenanthrene	92.8	8, b1	3	0
St 28	9-(1-hydroxyethyl) phenanthrene	104.1	8, b1	4	0
St 29	Phenanthrene-9-carboxylic acid	84.5	2, b1	3	0
*St 30	3-acetoxy-4, 6-phenanthrylene oxide	94.7	2, b1	3	0
St 31	3-(1-hydroxy-ethyl) phenanthrene	0	0	0	0
St 32	3-[2-(diethylamino)-1-hydroxy-ethyl] phenanthrene	0	0	0	0
St 33	9-[2-(dimethylamino)-1-oxo-ethyl] phenanthrene hydrochloride	26.9	1, b1	6	0
St 34	3-phenanthryl-acetic acid	9.7	0	0	0
St 35	9-(9-phenanthryl) acrylic acid methyl ester	98.8	6, b1	2	0
St 36	9-(9-phenanthryl)-propionic acid	74.3	0	5	0
St 37	9-(9-phenanthryl) propionic acid methyl ester	89.9	2, b1	4	0
St 38	9-(3-phenanthryl) acrylic acid ethyl ester	87.9	4, b1	3	0
St 39	9-(3-phenanthryl)-propionic acid	73.2	1, b1	5	0
St 40	9-(3-phenanthryl) propionic acid methyl ester	95.3	1, b1	5	0
St 41	9-(3-phenanthryl)-propionic acid hydrazide	89.3	1, b1	5	0

¹ Footnote 1 of table 1 applies here except that sucrose was used at 40 gm. per liter; footnotes 2 to 5 of table 1 apply also to this table.

TABLE 3.—Effect of some phenanthrene derivatives (at 100 mg. per liter) on growth, sporulation, and starch formation by *Aspergillus niger* at 35° C. for 4 days in a dibasic optimum solution—Continued

Compound No.	Phenanthrene derivative (100 mg. per liter, or 0.01 percent)	Yield	Sporulation	Starch in—	
				Mycelium	Solution
		Percent			
St 42	β -(2-phenanthryl) acrylic acid	105.0	8, b1	3	0
*St 43	β -(2-phenanthryl)-propionic acid	42.1	0	5	0
St 44	β -(2-phenanthryl)-propionic acid hydrazide	17.4	0	5	1
St 45	Phenanthrene-9-carboxylic acid dimethylamide	77.2	6, b1	4	0
St 46	Phenanthrene-3-carboxylic acid dimethylamide	67.5	2, b1	5	0
St 47	9-aminomethyl-phenanthrene hydrochloride	0	0	0	0
St 48	Phenanthrene-3-carboxylic acid- β -(diethylamino)-ethyl-ester	84.5	2, b1	5	0
St 49	Phenanthrene-9-carboxylic acid- β -(diethylamino)-ethyl-ester hydrochloride	75.4	1, b1	4	0
St 50	2- ω -bromo-acetyl phenanthrene	103.6	6, b1	2	0
*St 51	3- ω -bromo-acetyl phenanthrene	4.8	1, b1	0	0
*St 52	2-[3-(1,2,3,4-tetrahydroisoquinolino)-1-oxo-propyl] phenanthrene hydrochloride	97.6	8, b1	1	0
*St 53	3-(3-piperidino-1-oxo-propyl) phenanthrene hydrochloride	108.9	8, b1	5	0
*St 54	3-[3-(1,2,3,4-tetrahydroisoquinolino)-1-hydroxy- α -propyl] phenanthrene hydrochloride	1.9	0	0	0
St 55	9-acetyl-8-octahydrophenanthrene	94.3	2, b1	4	0
St 56	1,2,3,4,5,6,7,8-octahydrophenanthrene-9-carboxylic acid methyl ester	101.0	8, b1	4	0
St 57	8-octahydrophenanthrene-9-carboxylic acid	78.2	2, b1	5	0
St 58	3-ethoxy-4-acetylaminophenanthrene	95.3	4, b1	5	0
St 59	9-ethoxy-10-acetylaminophenanthrene	97.1	6, b1	3	0
St 60	2-propionyl-9,10-dihydro-phenanthrene	104.8	7, br	3	0
St 61	9,10-dihydrophenanthrene-2-carboxylic acid	38.6	0	5	0
St 62	2-[2-(diethylamino)-1-acetoxy- α -propyl]-9,10-dihydrophenanthrene hydrochloride	0	0	0	0
St 63	2-[2-(dimethylamino)-1-hydroxy- α -propyl]-9,10-dihydrophenanthrene hydrochloride	0	0	0	0
St 64	2-[2-(diethylamino)-1-acetoxy- α -propyl]-9,10-dihydrophenanthrene hydrochloride	9	0	0	0
*St 65	3-hydroxyphenanthrene-4-aldehyde	90.0	2, b1	4	0
*St 66	γ -(1-naphthyl)- α -butyric acid	1.0	0	0	0
St 67	γ -(2-naphthyl)- α -butyric acid	9.0	0	0	0
*St 68	4-hydroxyphenanthrene	0	0	0	0
*St 69	1-hydroxyphenanthrene	0	0	0	0
St 70	2-hydroxyphenanthrene (2-phenanthrol)	0	0	0	0
St 71	Oxazole or 3-hydroxy-4-aminophenanthrene	101.9	8, b1	3	0
*St 72	3-hydroxy-6-[2-(diethylamino)-1-hydroxy-ethyl] phenanthrene hydrochloride	0	0	0	0
*St 73	3-hydroxy-6-[2-(diethylamino)-1-acetoxyethyl] phenanthrene hydrochloride	89.3	2, b1	2	0
*St 74	3-acetoxy-6-[2-(diethylamino)-1-acetoxyethyl] phenanthrene hydrochloride	83.3	4, b1	3	0
St 75	3-dimethylamino-4-hydroxy-1,2,3,4-tetrahydrophenanthrene hydrochloride	84.3	2, b0	4	0
St 76	2-diethylamino-1-hydroxy-1,2,3,4-tetrahydrophenanthrene hydrochloride	87.2	4, b1	5	0
St 77	3-dimethylaminomethyl-4-oxo-1,2,3,4-tetrahydrophenanthrene hydrochloride	93.4	2, b1	5	0
St 78	3-(dimethylaminomethyl)-4-acetoxy-1,2,3,4-tetrahydrophenanthrene hydrochloride	98.5	4, b1	4	0
St 79	3-(dimethylaminomethyl)-4-hydroxy-1,2,3,4-tetrahydrophenanthrene hydrochloride	91.3	4, b1	4	0
St 80	3-diethylaminomethyl-4-hydroxy-1,2,3,4-tetrahydrophenanthrene hydrochloride	78.9	4, b1	4	0
St 81	3-[(1,2,3,4-tetrahydroisoquinolino)methyl]-4-hydroxy-1,2,3,4-tetrahydrophenanthrene hydrochloride	102.4	8, b1	3	0
St 82	1-dimethylamino-1,2,3,4-tetrahydrophenanthrene hydrochloride	82.7	4, b1	4	0
*St 83	3-hydroxyphenanthrene	0	0	0	0
*St 84	9-aminophenanthrene	3.2	1, b1	0	0
*St 85	2-aminophenanthrene	0	0	0	0

The effect of position of substituents in the phenanthrene molecule is illustrated in certain cases (8). An oxo group was perceptibly more toxic in the 4 position (St 2) than in the 1 position (St 1). Phenanthrene with a ω -bromo-acetyl group in the 3 position (St 51) was more toxic than with the same group in the 2 position (St 50). Toxicity of 9-aminophenanthrene (St 84) was somewhat less than that of 2-amino-

phenanthrene (St 85). Toxicity (table 4) of the hydroxyphenanthrenes (St 68, St 69, St 70, St 83) appeared somewhat greater with phenolic hydroxyl in the 3 position than in the 1, 2, or 4 position.

Experiments with those phenanthrene derivatives sufficiently toxic to prevent germination at a concentration of 100 mg. per liter were repeated at a level of 10 mg. per liter. The results are shown in table 4. Compound St 20 was omitted unintentionally. Derivatives St 68, St 69, St 70, and St 83 were still toxic and barely permitted germination to take place. All 4 compounds were mono hydroxyphenanthrenes. Of the 12 other compounds tabulated in table 4 a total of 8 were amino derivatives, and 2 hydroxy. None of these compounds led to an increase in starch production at a concentration of 10 mg. per liter, with the possible exception of derivatives St 31 and St 32.

TABLE 4.—Effect of the more toxic phenanthrene derivatives at 10 mg. per liter on growth, sporulation, and starch formation by *Aspergillus niger* at 35° C. for 4 days in a dibasic optimum solution¹

Phenanthrene derivative (10 mg. per liter, or 0.001 percent)	Low sucrose (80 percent of optimum)				Phenanthrene derivative (10 mg. per liter, or 0.001 percent)	Low sucrose (80 percent of optimum)			
	Yield	Sporu- lation	Starch in—			Yield	Sporu- lation	Starch in—	
			Myce- lium	Solu- tion				Myce- lium	Solu- tion
	<i>Percent</i>					<i>Percent</i>			
Control	100.0	8, b1	3	0	St 63	98.9	8, b1	2	0
St 2	92.6	4, b1	3	0	*St 64	92.1	6, b1	3	0
St 9	71.6	2, b1	3	0	*St 68	.1	0	0	0
St 19	99.9	8, b1	3	0	*St 69	.3	0	0	0
*St 20					St 70	.1	0	0	0
St 31	97.0	4, b1	4	0	*St 72	85.5	2, b1	3	0
St 32	89.6	4, b1	4	0	*St 83	0+	0	0	0
St 47	101.8	8, b1	1	0	*St 85	86.1	1, b1	2	0
St 62	64.9	4, b1	1	0					

¹ See table 3, footnote 1; footnotes 2 to 5 of table 1 apply also to this table.

DISCUSSION

Though under conditions of extreme toxicity the yield, sporulation, and starch formation of *Aspergillus niger* were all diminished, this was not the case at intermediate stages. Response within this range appeared to depend upon the constitution of the toxic substance; and yield, sporulation, and starch varied independently of each other. In some instances growth was but slightly, whereas sporulation was greatly, diminished. In other cases growth and reproduction were injured in equal degree. Both types of response might be accompanied by either an increase or a decrease in starch content. However, no instance was found of a decrease in growth without a corresponding decrease in spore formation. Malachite green appeared to be most toxic of the substances tested and prevented germination at 1 p. p. m.

Addition of none of the compounds led to significant increase in yield over that of the control with the exception of 3-(3 piperidino-1-oxo-propyl) phenanthrene hydrochloride (St 53) and 2-propionyl-phenanthrene (St 8), which produced yields of 108.9 percent and 107.0 percent, respectively. These increases also were probably without significance. Definite increases in yield over that of the control

occurred only with solutions deficient in trace elements, and therefore under conditions in which the inorganic impurities present in the organic compounds could function. The increases were well defined under these conditions and satisfied the criteria of "chemical stimulation." It can be said, therefore, that none of the organic compounds were capable of leading to an increase in yield in an optimum solution for growth.

Starch formation was increased through a partial deficiency of trace elements and decreased by partial nitrogen deficiency. An increase in starch may be brought about by an improvement in nutrition (increase in yield) or through toxicity.

Instances of a diminution in growth accompanied by increased starch formation were given by acridine orange, acridine yellow, alizarine blue (Ehrlich), meldola blue, methylene blue, rhodamine B, rhoduline GO, and phenanthrene derivatives St 1, St 6, St 21, St 33, St 36, St 43, St 44, St 46, St 57, and St 61. Increased starch formation was accompanied in each case by a decrease in sporulation as well as yield. Eosin W gelb, erythrosin bluish, fuchsine S, indigo carmine, phosphine, and phenanthrene derivatives St 14, St 40, St 53, and St 58 gave increases in starch formation without an adverse effect on yield. No fixed relation between spore and starch formation occurred when yields remained maximum. On the other hand no decrease in yield occurred, though formation of starch did not take place, through the action of aniline blue, brilliant alizarine, ethyl red, fluorescein, naphthol green, orange G, quinoline yellow, tartrazine, acridine, benzidine sulfate, and 1, 2, 5, 6-dibenzanthracene. Caffeine and theobromine did not increase starch in *Aspergillus*, though they are reported by Ciamician and Ravenna (3) to do so in green plants.

Tests with N/20 iodine would indicate the presence of several constituents in the hyphal cell walls. The mycelium may absorb none of the iodine solution; an immediate brilliant blue color may be formed; or a brown spot may be formed that may or may not become blue later. It is assumed on the basis of these tests that the cell walls comprise a colorless ground stroma similar to that seen under starvation conditions, chitin which causes the brown reaction, and starch. Infiltration with fatty substance may also enter as a factor.

SUMMARY

The effects of 37 biological stains, 21 miscellaneous organic compounds, and 85 phenanthrene derivatives on growth, sporulation, and starch formation of *Aspergillus niger* were studied at concentrations of 1, 10, 20, and 100 mg. per liter. An increase in yield above that of the control occurred only in a solution partially deficient in trace elements and was attributed to the presence of inorganic impurities in the organic compounds. Toxicity was greatest with malachite green (1 p. p. m.), though the hydroxyphenanthrenes (10 p. p. m.) were also quite toxic. Responses in yield, sporulation, and starch formation were not uniform, but were related to the chemical constitution of the compound. Starch formation was decreased by low nitrogen and by certain compounds. Low trace elements led to an increase in starch formation, as did also certain relatively toxic compounds.

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HEMICELLULOSES OF ALFALFA HAY ¹

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INTRODUCTION

Although the literature dealing with the chemical composition of forage plants and hays is extensive, little information is available on the hemicelluloses from these plants and plant materials. Furthermore, many of the analytical data on the hemicelluloses recorded in the literature were obtained by methods now known to give erroneous results. Thus many investigators determined the hemicellulose content by subjecting the plant material (which in many cases had not been previously freed of pectin) to hydrolysis with dilute hydrochloric or sulfuric acid, determining the copper reduction value of the hydrolysate, and calculating the result as percentage of dextrose. Others have used the yield of furfural obtained by distilling the plant material with 12-percent hydrochloric acid as a measure of the hemicellulose content. Both these methods give results that are in effect the sum of several factors of unequal magnitude. Thus, on the one hand, the copper-reduction values of the several hexoses, pentoses, *d*-glucuronic acid, and *d*-galacturonic acid vary considerably, and, on the other hand, the yields of furfural afforded by arabinose, xylose, and the hexuronic acids differ greatly. Among the investigators who have used the analytical methods referred to above are Nelson (15),² Steinmetz (31), Leukel (14), and Albert (1).

A new approach to the study of the hemicelluloses has been made in recent years by a group of English investigators, among whom may be mentioned Schryver and his coworkers (10, 30), O'Dwyer (21, 22, 23), Norris (3, 4, 20), Preece (25, 26, 27), Norman (17, 18, 19), and Buston (7, 8, 9). As a result of their work our knowledge of the chemistry of these complex carbohydrates has been considerably increased. New methods of fractionation and separation have been introduced, and in addition it has been demonstrated that the hemicelluloses are not simply hexosans or pentosans or mixtures of these sugar anhydrides, but that they nearly always contain a uronic acid, which may be either *d*-glucuronic acid or *d*-galacturonic acid.

Employing the technique of extraction and fractionation introduced by O'Dwyer (21, 22, 23), by Norris and coworkers (3, 4, 20), and by Preece (25, 26, 27), Burkhart (6) isolated two hemicelluloses from alfalfa roots. On hydrolysis both fractions yielded *d*-glucose and *d*-xylose. A uronic acid was also present, but was not definitely identified.

The object of the investigation reported in this paper was to determine the character and composition of the hemicelluloses of alfalfa hay by methods recently developed.

¹ Received for publication November 18, 1939.

² Italic numbers in parentheses refer to Literature Cited, p. 779.

METHODS AND RESULTS

The alfalfa (*Medicago sativa*) hay used in this investigation was grown at the United States Department of Agriculture Beltsville Research Center. Analysis³ showed that it had the following constituents (all results were calculated on material oven-dried at 105° C.): Ash, 8.85 percent; alcohol-benzene extractives, 11.82 percent; furfural, 7.80 percent; uronic acids (as anhydride), 11.66 percent.

The alfalfa was cut into pieces about 1 inch long and extracted for about 30 hours with a 1:2 ethanol-benzene solution. The extracted material was placed in a large evaporating dish, and the residual ethanol-benzene evaporated on the steam bath.

EXTRACTION WITH 0.5-PERCENT AMMONIUM OXALATE SOLUTION

To 600 gm. of ground and ethanol-benzene-extracted alfalfa (563 gm. of moisture-free material), 4,500 cc. of 0.5-percent ammonium oxalate solution was added, and the mixture was digested at 85° C. for 24 hours. From time to time the mixture was stirred manually. The reaction mixture was filtered, and the digestion with ammonium oxalate solution was repeated three times. The combined filtrate from the four extractions was concentrated under reduced pressure to approximately one-fourth of its original volume, and four volumes of 95-percent ethanol was added to it. The precipitate was allowed to settle, was filtered, and washed with ethanol of graded strengths.⁴ The yield of moisture-free material amounted to 94.1 gm. (16.7 percent, calculated on the ethanol-benzene-extracted, oven-dried alfalfa). An amorphous grayish product was obtained.

In another experiment 600 gm. of ethanol-benzene-extracted alfalfa was first digested with water at 85° C. for 24 hours and then with 0.5-percent ammonium oxalate solution as described above. The aqueous and the ammonium oxalate extracts were concentrated under reduced pressure, and then each was treated with four volumes of 95-percent ethanol. The crude water-soluble and ammonium oxalate-soluble pectins were filtered off and washed with ethanol of graded strengths. The yields of the water-soluble and the ammonium oxalate-soluble pectins amounted to 23.6 and 72.7 gm., respectively, of moisture- and ash-free material.

To 359 gm. of pectin-free material (equivalent to 340 gm. of moisture-free material) a sufficient quantity of alcoholic sodium hydroxide solution (20 gm. of sodium hydroxide, 400 cc. of water, and sufficient 95-percent ethanol to make 1 liter of solution) was added to cover completely the extracted alfalfa, and the mixture was then digested at room temperature for 24 hours. From time to time the reaction mixture was stirred manually. The plant material was filtered off, and the digestion with alcoholic sodium hydroxide solution was repeated three times. The combined extract was neutralized with hydrochloric acid, and the ethanol was distilled off under reduced pressure. The residual solution was made acid with hydrochloric acid, and the precipitated lignin was filtered off and dried in vacuo at 56° C. over P₂O₅. The yield was 28 gm. The lignin obtained was

³ Ash was determined by igniting the sample in an electric muffle at 600° C. The ethanol-benzene extractives were determined by extracting the material with a 1:2 ethanol-benzene solution for 30 hours in a Soxhlet extractor. Furfural was determined by the Tollens-Kröber procedure as described by the Association of Official Agricultural Chemists (5). The uronic acids were determined according to the procedure recommended by Dickson, Otterson, and Link (11), as modified slightly by Phillips, Goss, and Browne (24).

⁴ The term "graded strengths" as used in this paper means 70-percent, 85-percent, 95-percent, and absolute ethanol.

light brown. When distilled with 12-percent hydrochloric acid it yielded furfural equivalent in weight to 0.5 percent of the weight of the lignin. The filtrate from the lignin precipitate was neutralized with sodium hydroxide solution and evaporated to dryness on the steam bath, and the residual material was dried in the oven at 105° C. When distilled with 12-percent hydrochloric acid, this material gave negligible quantities of furfural, thus indicating that this method of delignification did not bring about any appreciable loss of hemicelluloses.

ISOLATION OF THE HEMICELLULOSES

The material that had been extracted with alcoholic sodium hydroxide solution was placed on the steam bath, and the ethanol was removed by evaporation. It was then mixed with sufficient 5-percent aqueous sodium hydroxide solution to make a thin suspension and was allowed to digest at room temperature for 24 hours. The reaction mixture was stirred from time to time. At the end of the 24-hour period, the mixture was filtered, and the digestion of the residual material with 5-percent aqueous sodium hydroxide solution was repeated three times. When the combined extracts were acidified with acetic acid, only a slight turbidity was produced, thus indicating the absence of any appreciable quantities of hemicellulose fraction A according to the classification and nomenclature of Norris and his coworkers (3, 4, 20).

To the solution that had been made acid with acetic acid one-half its volume of acetone was added. On standing, a precipitate settled out. This was separated with the aid of the centrifuge. The product was washed with ethanol of graded strengths, and after centrifuging, the wash ethanol was removed by decantation. The product was dried in the desiccator over calcium chloride. The yield of this fraction, which corresponded to the B fraction according to the classification of Norris and his coworkers, amounted to 8.0 percent of the moisture-free and ethanol-benzene-extracted material.

A small portion of the filtrate from the above-described hemicellulose preparation, to which another one-half volume of acetone was added, failed to yield any precipitate, thus indicating the absence of any C fraction. However, when 1 volume of 95-percent ethanol was added, some precipitate settled out. This precipitate was separated with the aid of the centrifuge and washed with ethanol of graded strengths. The yield amounted to 1.9 percent (calculated on the basis of the moisture-free product and the moisture-free and ethanol-benzene-extracted starting material). This product, which may be designated as the D fraction, contained 6.88 percent of uronic acids (as anhydrides) and when distilled with 12-percent hydrochloric acid afforded 20.38 percent of furfural. Owing to lack of sufficient material, this product was not examined further.

PURIFICATION OF HEMICELLULOSE FRACTION B

Fraction B was purified by the method of Salkowski (28). The hemicellulose was dissolved in 4-percent aqueous sodium hydroxide solution, the solution was filtered through glass wool, and the filtrate poured into an excess of Fehling's solution. The bulky and gelatinous hemicellulose-copper complex was filtered off, and dissolved in dilute hydrochloric acid solution. An equal volume of acetone was then added. The precipitated hemicellulose was filtered off and washed

with ethanol of graded strengths. It was dried in the Abderhalden drier over P_2O_5 at $56^\circ C$. The yield amounted to 4.3 percent of the moisture-free and ethanol-benzene-extracted alfalfa. Nothing definite was isolated from the filtrate of the hemicellulose-copper complex.

An analysis of the purified B hemicellulose fraction gave the following results (calculated on the moisture- and ash-free basis): Nitrogen, 1.03 percent; total furfural (calculated as pentosans), 77.34 percent; uronic acids (as anhydrides), 12.13 percent; methoxyl, 2.27 percent.

HYDROLYSIS OF HEMICELLULOSE FRACTION B

The hemicellulose (14.5 gm.) was treated with 725 cc. of 2.5-percent sulfuric acid, and the mixture was boiled under the reflux condenser for 6 hours. The dark insoluble material was filtered off. When dried at $105^\circ C$. it amounted to 0.88 gm. The filtrate was partly neutralized with a solution of barium hydroxide, and the neutralization was completed with barium carbonate. The barium sulfate and the excess of barium carbonate were filtered off and washed with hot water, and the washings were added to the main filtrate. The filtrate was clarified with Norit and with Filter Cel and concentrated under reduced pressure to a volume of approximately 100 cc. at a temperature not exceeding 50° . To the concentrated solution was added five times its volume of 95-percent ethanol, and the precipitated barium salt was filtered off. When dried in the Abderhalden drier over P_2O_5 at $56^\circ C$., this salt weighed 1.26 gm. The filtrate from the barium salt was concentrated under reduced pressure to a thin sirup, and 5 volumes of alcohol was added to it. The solution, which became only slightly turbid, was filtered and concentrated in vacuo to a thin sirup. On standing, crystals separated out and were filtered off and recrystallized from 70 to 75-percent ethanol. The crystals were identified as *d*-xylose by specific rotation, by Bertrand's (13, p. 58) method, and by their optical properties.⁵ The yield amounted to 10 gm.

A benzylphenylhydrazone and diphenylhydrazone were prepared from the sirup from which xylose had separated. They were identified by their melting points as the hydrazones of *l*-arabinose. The optical properties of the diphenylhydrazone agreed exactly with those of a known specimen of *l*-arabinose diphenylhydrazone.

By means of the Wise and Peterson (33) modification of the Neuberg and Wohlgemuth (16) method for the quantitative estimation of arabinose, it was found that the B hemicellulose fraction contained 0.8 percent of arabinose.

The sirup was also tested for glucose, fructose, mannose, and galactose, with negative results.

The barium salt (1.26 gm.) that had been precipitated from the sugar solution with 95-percent ethanol gave Tollens' (32) naphthoresorcinol test for uronic acid. It gave no music acid on oxidation with nitric acid, and also with bromine water, thus indicating that it was not galacturonic acid. The *p*-bromophenylosazone was prepared according to the procedure of Goldschmiedt and Zerner (12). The osazone melted with decomposition, but the decomposition point (184°) was considerably lower than that recorded by Goldschmiedt and Zerner. Although it seems probable that the uronic acid ob-

⁵ All identifications by optical methods were made by G. L. Keenan of the Microanalytical Division of the Food and Drug Administration, U. S. Department of Agriculture.

tained was *d*-glucuronic acid, nevertheless, in view of the lower decomposition point of the isolated *p*-bromphenylosazone of the barium salt, this cannot be stated with certainty. The possibility that it may have been one of the methyl ethers of *d*-glucuronic acid is not excluded, especially since the barium salt contained 1.4 percent of methoxyl. Sands and Gary (29), as well as Anderson, Russell, and Seigle (2), found it difficult to identify the uronic acid in the presence of the methoxyl group.

SUMMARY

The hemicelluloses of alfalfa hay were isolated. When fractionated according to the method of Norris and his coworkers, they were found to consist almost entirely of the B fraction. On hydrolysis this product yielded *d*-xylose, together with a small amount (0.8 percent) of *l*-arabinose. This fraction contained also a uronic acid that was probably glucuronic acid or one of its methyl derivatives.

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HEMICELLULOSES OF WHEAT STRAW¹

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INTRODUCTION

In 1890, 2 years before the term "hemicellulose" was introduced into chemical literature by Schulze (12),² Allen and Tollens (1) extracted wheat straw with alkali and obtained a product that on hydrolysis afforded *d*-xylose. In 1901 Salkowski (11) extracted wheat straw with boiling 6-percent sodium hydroxide solution and showed that the xylan could be precipitated from the alkaline extract by means of Fehling's solution and thus freed of certain impurities. Heuser, Braden, and Kürschner (6) improved Salkowski's method, and in order not to contaminate their product with lignin, they used bleached straw pulp (method of delignification not given) as their starting material. They obtained a product that was 96 percent xylan.

The investigation reported in this paper was undertaken for the purpose of determining the character and composition of the hemicelluloses of wheat straw by methods recently developed.

METHODS AND RESULTS

Analysis showed that the wheat (*Triticum aestivum*) straw used in this investigation had the following constituents: ³ Ash, 4.37 percent; ethanol-benzene extractives, 6 percent; pentosans,⁴ 29.4 percent; uronic acids (as anhydride), 4.5 percent; pectin (as calcium pectate), 1 percent.

The straw was cut into small pieces and extracted for about 30 hours with a 1:2 ethanol-benzene solution in a large Soxhlet-type extractor. The extracted material was heated on the steam bath until free of solvent and then ground in a Wiley mill until it was fine enough to pass a 60-mesh sieve. The extracted straw was treated with hot (85° C.) water in the proportion of 1 liter of water to 100 gm. of straw, and the mixture was digested at 85° for 4 hours. The straw was filtered off, and the extraction with hot water was repeated twice. The combined aqueous extract was concentrated under reduced pressure and treated with four volumes of 95-percent ethanol. The precipitate was then filtered off. After drying it amounted to 2.1 percent of the weight of the dry ethanol-benzene-extracted straw.

The straw that had been extracted with hot water was then extracted with 0.5-percent aqueous ammonium oxalate solution at 85° C., and the procedure described above for the hot-water extraction was followed, except that 95-percent ethanol that had been acidified with hydrochloric acid was used for the precipitation. The precipitate

¹ Received for publication November 18, 1939.

² Italic numbers in parentheses refer to Literature Cited, p. 785.

³ The analytical methods used were those described by Phillips and Davis (10).

⁴ Unless indicated otherwise the percentages of pentosans recorded in this paper have not been corrected for the furfural afforded by the uronic acid.

was filtered off and dried in vacuo over sulfuric acid. The yield amounted to 3.1 percent of the weight of the dry ethanol-benzene-extracted straw.

ISOLATION OF THE HEMICELLULOSES

To 500 gm. (moisture-free) of the straw that had been successively extracted with ethanol-benzene solution, hot water, and ammonium oxalate solution, 4.5 liters of 5-percent aqueous sodium hydroxide solution was added. The mixture was digested at room temperature for 24 hours, being stirred by hand from time to time. It was then filtered, and the digestion with 5-percent aqueous sodium hydroxide solution was repeated four times. The cellulose residue from the last extraction operation was washed with water and dried at 105° C. It weighed 270.7 gm.

The combined alkaline extract was treated with an equal volume of 95-percent ethanol, and after being thoroughly mixed was allowed to stand at room temperature for 24 hours. The supernatant liquid was then drawn off, and the hemicelluloses were freed of sodium hydroxide by repeated washing with 95-percent ethanol. The product was suspended in 70-percent ethanol, and concentrated hydrochloric acid was added to the mixture until it was distinctly acid. After the product had stood overnight, the supernatant liquid was drawn off, and the hemicelluloses were repeatedly washed with 70-percent ethanol until free of sodium chloride. It was then washed with ethanol of graded strengths⁵ and finally with anhydrous ether. All the washings were done by thoroughly mixing the hemicelluloses with the solvent in centrifuge bottles and then separating the supernatant liquid with the aid of the centrifuge. The yield amounted to 140.0 gm. The analytical data on this product are as follows: Ash, 5.59 percent; lignin, 3.27 percent; uronic acid (as anhydride), 5.07 percent; total furfural (calculated as pentosans), 80.51 percent.

DELIGNIFICATION OF HEMICELLULOSES

The delignification was accomplished by a modification of the procedure used by Van Beckum and Ritter (14) for the preparation of holocellulose. Two 56-gm. portions of the crude hemicelluloses were placed in two centrifuge bottles (400-cc. capacity) each containing 56 cc. of water. Each centrifuge bottle was provided with a stopper through which passed an inlet tube extending nearly to the surface of the hemicellulose preparation, and an outlet tube. The hemicelluloses were thoroughly mixed with the water, and the centrifuge bottles were placed in a bath of ice water. A slow stream of chlorine gas was passed through each bottle for 1 hour. Every 6 to 8 minutes the stoppers in the bottles were removed and the contents stirred. At the end of the chlorination period, 300 cc. of 95-percent ethanol was added to each bottle. The contents were thoroughly mixed and then centrifuged. The supernatant ethanol solution was drawn off, and the chlorinated material in each centrifuge bottle was treated with 300 cc. of a 3-percent ethanolamine solution in 95-percent ethanol, and after being mixed was heated in a water bath at 80° C. for 10 minutes. The reaction mixture was allowed to cool to 50° and then was centrifuged. The supernatant liquid was drawn off,

⁵ The term "graded strengths" as used in this paper means 70-percent, 85-percent, 95-percent, and absolute ethanol.

and the extraction with ethanolamine was repeated. The extracted hemicelluloses were then washed with 95-percent ethanol and finally with absolute ethanol and with ether. The material was dried in the Vacuum desiccator over concentrated sulfuric acid. The product was colorless and weighed 115.6 gm. The analytical data on the material are: Ash, 1.78 percent; lignin, trace; uronic acid (as anhydride), 5.73 percent; furfural (calculated as pentosans), 83.21 percent.

FRACTIONATION OF THE HEMICELLULOSES

One hundred grams of the hemicelluloses, delignified as described above, was dissolved in a solution containing 60 gm. of sodium hydroxide in 2,250 cc. of water. The alkaline solution was filtered through glass wool, and the filtrate was made slightly acid with acetic acid. Since a preliminary experiment had indicated that fraction A (classification and nomenclature of Norris and Preece (2, 3, 9) was present in only small amounts, no attempt was made to separate this fraction. Accordingly, one-half its volume of acetone was added to the acidified solution, and the hemicellulose fraction B together with the small quantity of fraction A was separated with the aid of the centrifuge. The product was washed with ethanol of graded strengths and finally with anhydrous ether. It was dried in the vacuum desiccator over sulfuric acid. The yield amounted to 79.2 gm.

To the filtrate from the previous hemicellulose fraction 95-percent ethanol was added until no more precipitate settled out. This hemicellulose fraction (fraction C) was removed by centrifuging. It was washed with graded strengths of ethanol and finally with anhydrous ether. It was dried in vacuo over sulfuric acid. The yield was 9.0 gm. The analytical data on the material are: Ash, 3.66 percent; uronic acid (as anhydride), 9.64 percent; pentosans, 70.58 percent; methoxyl, 3.74 percent.

It will be observed that the percentages of uronic acid and methoxyl were considerably higher in this hemicellulose fraction than in the A and B fractions. Because of the limited quantity of material available, this fraction was not hydrolyzed.

HYDROLYSIS OF THE COMBINED A AND B HEMICELLULOSE FRACTIONS

To 35 gm. of the hemicellulose preparation, 750 cc. of 2.5-percent sulfuric acid was added, and the mixture was heated on an electric hot plate under a reflux condenser for 15 hours. The reaction mixture was allowed to cool and then was filtered on a weighed filter paper. After drying at 105° C., the insoluble residue amounted to 0.3426 gm. Approximately nine-tenths of the calculated quantity of barium hydroxide solution was added to the filtrate slowly with stirring, while the temperature of the reaction mixture was kept at 40°. An excess of barium carbonate was then added, and the mixture was heated at 70° to 80° for 20 minutes while it was continually stirred. To this mixture 5 gm. of Norit and 3 gm. of Filter Cel were added, and the heating was continued for another 15 minutes. After the mixture had stood overnight, the clear supernatant liquid was drawn off and filtered. The sediment of barium sulfate, barium carbonate, etc., was transferred to centrifuge bottles and centrifuged, and the clear supernatant liquid was drawn off. The residue was

mixed with distilled water and centrifuged, and the supernatant liquid was separated from the sludge. The clear solutions from the two centrifuging operations were combined, filtered, and added to the main filtrate. This solution was concentrated under reduced pressure to a volume of approximately 100 cc. (the temperature of the bath during concentration did not exceed 50°). The concentrated solution was poured into four volume of 95-percent ethanol, and the resulting mixture was centrifuged. The supernatant liquid was drawn off, and the precipitate of the barium salt was washed with absolute ethanol and with anhydrous ether and was finally dried in vacuo over sulfuric acid. The product weighed 0.5 gm.

The supernatant liquid from the barium salt was concentrated under reduced pressure at 50° C., and the thin sirup was again treated with 4 volumes of ethanol. As no more precipitate of barium salt was obtained, the sugar solution was again concentrated under reduced pressure to a thin sirup, and this was allowed to stand in the vacuum desiccator until it started to crystallize. After an appreciable quantity of sugar had crystallized from the solution, the mixture was filtered. The sugar was recrystallized from dilute ethanol, and after it was dried in the vacuum desiccator over sulfuric acid it weighed 15.5 gm. This sugar was identified as *d*-xylose by the specific rotation and melting point and by Bertrand's method (5). The optical properties⁶ of the double cadmium salt prepared by Bertrand's method were identical with those of the salt prepared by the same method from a known specimen of pure *d*-xylose.

A diphenylhydrazone was prepared from the sirup from which *d*-xylose had separated. It was identified as the diphenylhydrazone of *l*-arabinose by its melting point and by the optical properties of the crystals.

Analysis by the Wise and Peterson (15) modification of the method of Neuberg and Wohlgemuth (8) showed that the sirup contained 1.27 gm. of *l*-arabinose. The total reducing sugars (calculated as glucose), determined by the Munsen-Walker procedure, amounted to 10.97 gm. The sirup was also tested for glucose, mannose, and galactose, but with negative results.

The barium salt that had been precipitated from the sugar solution with ethanol gave Tollens' (13) naphthoresorcinol test for uronic acids. It gave no mucic acid on oxidation with nitric acid or on oxidation with bromine water, thus indicating that it was not galacturonic acid. An attempt was made to prepare the *p*-bromphenylosazone of glucuronic acid according to the procedure of Goldschmiedt and Zerner (4), but a brown amorphous product was obtained that did not appear to have the properties of the *p*-bromphenylosazone recorded by these investigators. Because of lack of sufficient material, it was not possible to try other means of identification, and the uronic acid was therefore not definitely identified.

The analytical data on the combined A and B hemicellulose fractions are as follows:⁷ Ash, 4.51 percent; nitrogen, 0.06 percent; lignin, none; methoxyl, 1.33 percent; uronic acid (as anhydride), 4.76

⁶ All identifications by optical methods were made by G. L. Keenan of the Microanalytical Division of the Food and Drug Administration, U. S. Department of Agriculture.

⁷ In calculating the yield of furfural from the percentage of uronic acid anhydride, the assumption was made that the uronic acid was *d*-glucuronic acid. According to Lefèvre and Tollens (7), *d*-glucuronic acid affords 19 percent of its weight of furfural. In calculating the percentages of furfural derived from *l*-arabinose and *d*-xylose, consideration was given to the fact that these sugars furnish 75 and 90 percent, respectively, of the theoretical yield of furfural.

percent; total furfural, 55.8 percent; furfural from uronic acid, 0.9 percent; *l*-arabinose, 3.6 percent; furfural from *l*-arabinose, 1.7 percent; furfural from *d*-xylose (by difference), 53.2 percent; *d*-xylose (calculated from furfural), 92.3 percent; molar ratio of uronic acid to *l*-arabinose and *d*-xylose, 1:0.9:23.

Dr. Sterling B. Hendricks of the Bureau of Plant Industry made an X-ray examination of two samples (Nos. 1 and 2) of the hemicelluloses of wheat straw. Sample No. 1 was prepared as described on pages 782 and 783 of this paper. Sample No. 2 was prepared as follows: The residual cellulosic material obtained in the preparation of sample No. 1 was exhaustively extracted with a 10-percent aqueous sodium hydroxide solution at ordinary room temperature. To the alkaline extract an equal volume of 95-percent ethanol was added, and the precipitated hemicelluloses were separated with the aid of the centrifuge. The product was freed of sodium hydroxide by repeated washing with 95-percent ethanol. The hemicellulose precipitate was then suspended in 95-percent ethanol, and concentrated hydrochloric acid was added to the mixture until it was distinctly acid. After the mixture had stood overnight, the supernatant liquid was drawn off, and the hemicelluloses were washed with ethanol of graded strengths and finally with anhydrous ether. The product was dried in the vacuum desiccator over concentrated sulfuric acid. On analysis this product gave the following results: Pentosans, 73.83 percent; uronic acids (as anhydride), 3.75 percent. Hendricks' report was as follows:

X-ray diffraction photographs were made from two samples of hemicelluloses from wheat straw. Samples for study were kneaded into small pellets which were mounted in the X-ray beam. Both materials gave the usual type of X-ray powder diffraction pattern characteristic of crystalline substances or randomly oriented fibers. However, diffraction patterns of the two samples differed greatly in the positions and intensities of the interference maxima. Fe K radiation was used in the X-ray work.

SUMMARY

The hemicelluloses of wheat straw were isolated, and the product was freed of nitrogen and lignin. When fractionated, the hemicelluloses were found to consist chiefly of the B fraction, together with a small proportion of the C fraction. On hydrolysis the B fraction afforded *d*-xylose, *l*-arabinose, and a uronic acid that was probably glucuronic acid or a methyl derivative of it. The molar ratio of uronic acid to *l*-arabinose and *d*-xylose in this fraction was found to be 1:0.9:23, respectively.

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JOURNAL OF AGRICULTURAL RESEARCH

VOL. 60

WASHINGTON, D. C., JUNE 15, 1940

NOV 1940
No. 12

STUDIES ON THE NATURAL INOCULATION OF SEED BARLEY WITH COVERED SMUT (*USTILAGO HORDEI*)¹

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INTRODUCTION

The present investigation is one in a series of studies by the writer (38, 39, 40, 42, 43, 44, 45)² on inoculation, incubation, and infection in the smuts of barley (*Hordeum vulgare* L.) and was undertaken with a twofold objective: (1) To obtain a better fundamental knowledge of the natural method of inoculation and the reasons for its effectiveness and (2) to determine the cause of the usual ineffectiveness of the artificial method of seed inoculation and its exceptional effectiveness as reported by Faris (11).

PREVIOUS INVESTIGATIONS

Since Jensen (23) first reported, in 1888, two kinds of barley smut—loose smut (*Ustilago nuda* (Jens.) Kell. and Sw.) and covered smut (*U. hordei* (Pers.) Kell. and Sw.)—it has been singularly difficult to acquire an accurate knowledge as to how these smuts initiate, develop, and complete their parasitic relation with the barley host. For example, after it had been thoroughly established, apparently, that the barley loose smut fungus produces infection only through inoculation of the flowers (4, 5, 7, 13, 17, 18, 21, 33), up to 100 percent of loose smut was obtained through inoculating the seed (48, 49). Furthermore, seed treatment with certain surface disinfectants frequently was found highly effective in control (30, 31, 46, 50, 51) after convincing tests apparently had proved that only a long and penetrating seed treatment could reach and control the deep-seated infection threads of this smut (2, 13, 22, 24). In 1932 the writer (38) first reported that barley loose smut in the United States is caused not only by *Ustilago nuda* but also by another widespread fungus for which the name *U. nigra* was proposed. It was found that *U. nigra* is amenable to control through seed treatment with certain surface disinfectants and is able to produce seedling infection and smutted plants when the spores are superficially applied to the seed (38, 39). This discovery clarified the loose smut situation.

The barley covered smut, however, still remained difficult to understand. For example, it has been generally accepted that the firm, compact covered smut heads remain intact while they are in the field and that the seed first becomes inoculated in threshing, through disintegration of the smut heads and the adherence of spores to the surface of the kernels (8, 10, 16, 19, 20, 22, 27, 34, 35, 37). If this were

¹ Received for publication April 15, 1940. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the New York (Cornell) Agricultural Experiment Station.

² Italic numbers in parentheses refer to Literature Cited, p. 808.

entirely true, then the treatment of seed with a surface disinfectant should kill the superficially borne spores and control the smut. A wealth of literature is available, however, which shows that numerous liquid and dust fungicides applied to seed from smutted fields frequently have failed to effect complete control (14, 28, 29, 31, 50, 51). Moreover, if spores on the surface of seed were accountable for the heavy outbreaks of covered smut so frequently observed in growers' fields, then the artificial dusting of seed with millions of spores should prove an effective method of inoculation. Extensive tests over many years, in this country and in other countries, have shown repeatedly that this method of inoculation usually results only in low percentages of covered smut (1, 3, 6, 23, 25, 32, 36, 47). The effect of this predicament on the progress of research has been well epitomized by Mackie (32):

Experiments in California have for three years failed in attempt to create heavy smut attacks by artificially inoculating seed barley. This has been the experience of other investigators. It has therefore been impossible to determine the smut resistance of any of the hundreds of barley varieties under test.

Adding further to the complexity of the situation, Faris (11), in diametric contrast to the usual experience, reported excellent results with the spore-dusting method of seed inoculation. There is nothing to indicate that the technique of inoculation used by Faris differed from that used by Mackie and others reporting unsatisfactory results with this method.

Pending the solution of the problem of inoculation noted above, covered smut has long continued an important hazard in barley culture in the United States. Annual losses estimated at 3 million to more than 5½ million bushels are not infrequent. In individual fields, 30 to 50 percent of covered smut has been reported.³ It is therefore evident that the natural method of seed inoculation, whatever it may be, is highly effective.

EXPERIMENTAL RESULTS

LOCATION OF INOCULUM ON NATURALLY INOCULATED SEED

Procedure in the present investigation was influenced by the outstanding reports of Zade (52, 53) and Gage (15) on the seedling-infecting smuts of oats. As with the covered smut of barley, it had long been considered that spores of the oat smuts remained dormant after reaching the seed and that germination of the spores did not take place until the seed was sown and began to germinate. Zade discovered, however, that in the floral-inoculating loose smut of oats, the spores normally begin to germinate soon after reaching the flowers and that mycelium from the spores constitutes an effective subhull inoculum that is accountable for most of the infection of seedlings and subsequent smutting of heads. Later Gage found that, with both loose and covered smuts of oats, spores that reach the seed may

³ UNITED STATES BUREAU OF PLANT INDUSTRY. ESTIMATE OF CROP LOSSES DUE TO PLANT DISEASES. 1917. U. S. Bur. Plant Indus., Plant Dis. Bul. 2: 1-18. 1918. [Mimeographed.]

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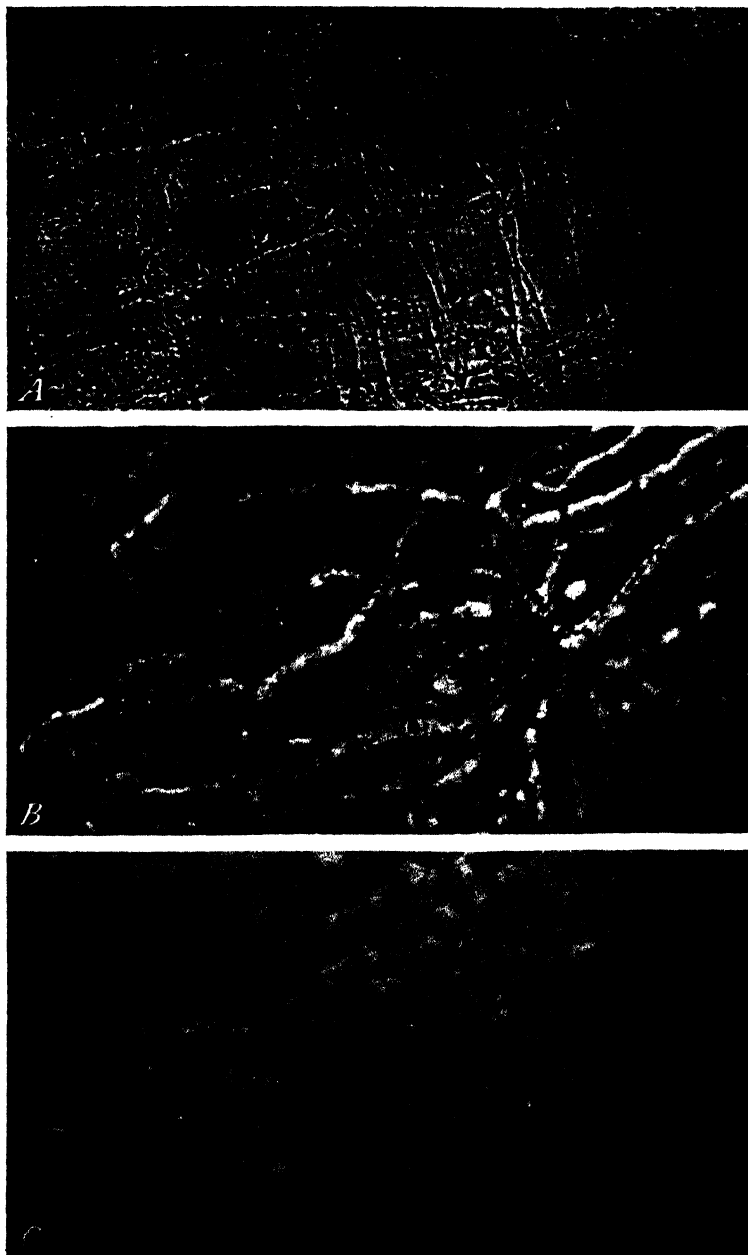
germinate not only during the blossoming period but also during the maturing period and even when the seed is in storage. Although differing with Zade in some details, Gage also found that inoculum beneath the hulls was largely responsible for most of the infection of seedlings and the subsequent smutting of heads. It seemed likely, therefore, that likewise in barley the most effective inoculum of covered smut, under natural conditions of inoculation, might be that which became lodged under the hulls of the seed. To determine whether this might occur, six hulled varieties of barley naturally inoculated with covered smut were studied. These barleys had been collected in Kansas, Maryland, Pennsylvania, Washington, and Wisconsin, from fields heavily infested with covered smut; all the fields, except that in Wisconsin, were practically free of loose smut. After being threshed and then stored in the growers' bins until the following late February or March, the seed was sent to the writer. During the 2 weeks preceding sowing, the hulls were carefully removed from some of the seed of each variety with the aid of a scalpel.

The experiment was designed also to throw light on two related phases. (1) Since the copper carbonate seed treatment frequently has been shown to be ineffective in the control of barley covered smut and the reasons for this have not been clearly understood, both normal and dehulled seed of the various lots were dusted with copper carbonate to determine the possible role of the hulls in protecting inoculum that might be carried beneath them. (2) In some previous studies with oats, dehulling the seed has been employed to determine not only the seed parts that harbor inoculum but also the approximate proportions of effective inoculum borne by the caryopsis and by the hulls. The writer (4) previously has shown that at least with oats this method is not reliable. The percentages of smutted plants from normal and dehulled seed maintain no consistent relation under different environmental conditions of seeding and plant growth. In order to determine whether this also might hold true with barley for covered smut, normal and dehulled seed, untreated or treated with copper carbonate, was sown at different times in the field and greenhouse at the Arlington Experiment Farm, Arlington, Va., and in the field at Ithaca, N. Y. (Cornell University). The results are presented in table 1.

It will be noted in table 1 that: (1) Plants from the dehulled seed showed, on an average, approximately three-fourths as much smut (24.9 percent) as the plants from seed with the hulls on (33.5 percent). This is evidence of the occurrence of subhull inoculum in seed lots from eastern, western, and central areas of the country and of the high degree of effectiveness of this inoculum under various field and greenhouse conditions; (2) the copper carbonate seed treatment completely controlled covered smut when applied to dehulled seed but not when applied to seed with hulls on. The frequently noted ineffectiveness of the copper carbonate seed treatment against barley covered smut thus would seem to be due to the fact that, in nature, inoculum may occur beneath the hulls and the hulls may protect this inoculum to some extent from the lethal action of the disinfectant.

TABLE 1.—Incidence of covered smut in barley as influenced by removing the hulls from naturally inoculated seed and sowing the dehulled and normal seed under various conditions, and the effectiveness of the copper carbonate seed treatment in control of covered smut when applied to normal and dehulled seed

Seed lot No.	Variety and source	Location of experiment	Date of seeding	Heads of barley grown from seed in indicated condition					
				Seed untreated			Seed treated		
				Hulls on		Hulls removed	Increase (+) or decrease (−) in smutted heads resulting from removal of hulls	Hulls on	
				Total heads	Smutted heads	Total heads		Total heads	Smutted heads
				Number	Percent	Number	Percent	Number	Percent
1	Stavropol (Kansas, 1934)	{ Greenhouse, Arlington, Va. { Field, Ithaca, N. Y.	March 1935	108	58.3	114	34.2	120	5.0
			April 1935	180	6.7	166	−41.3	138	133
2	Colless (Washington, 1934)	{ Greenhouse, Arlington, Va. { Field, Ithaca, N. Y.	March 1935	120	87.5	135	7.2	132	183
			April 1935	176	14.2	108	−16.2	116	0
3	White Club (Washington, 1934)	{ Greenhouse, Arlington, Va. { Field, Arlington, Va.	March 1936	168	97.6	93	−8.5	116	0
			do.	215	13.5	239	−29.5	5.4	−90.0
4	Alma (Pennsylvania, 1934)	{ Greenhouse, Arlington, Va. { do.	March 1935	114	52.6	87	−5.4	159	9.4
			do.	204	66.2	33	+44.3	141	0
5	Tennessee Winter (Maryland, 1934)	{ Field, Ithaca, N. Y. { Greenhouse, Arlington, Va.	April 1935	248	4.4	143	−31.3	201	7.5
			March 1935	104	38.5	60	+59.1	82	0
6	Wisconsin Pedigree No. 5 (Wisconsin, 1928)	{ do. { do.	November 1929	231	19.5	198	+47.3		
			March 1930	239	9.3	256	−1.0		
Total or average				2,127	33.5	1,632	−25.7		



A, Portion of a pericarp of Colless barley (seed lot 2, table 1) from a field heavily infested with covered smut ($\times 210$). *B*, Portion of the same pericarp, showing the ramifying, septate mycelium ($\times 940$). *C*, Another part of the same pericarp; the septate mycelium appears to have emanated from the covered smut spore indicated by arrow ($\times 940$).

A further analysis of the data of table 1 shows that the reduction in smutted heads resulting from dehulling the seed varied widely in the different lots sown under similar conditions and also in the individual lots sown under the different conditions of field and greenhouse. In 5 of the 12 tests, dehulling resulted not in a reduction but in an increase of covered smut. It seems evident, therefore, that in barley, as in oats, removal of the hulls from naturally inoculated seed does more than merely eliminate the inoculum that may be carried by the hulls. Evidently the hulls also exert some influence in tempering the environmental conditions surrounding the caryopsis, and their removal may lead to conditions that promote a greater or a lessened effectiveness in infection from the portion of inoculum that remains with the caryopsis. The amount of reduction in smut plants or heads resulting from removal of the hulls, therefore, cannot be used reliably to indicate the proportion of the inoculum carried by the hulls.

MICROSCOPIC STUDIES OF INOCULUM RESIDING WITH THE PERICARPS OF NATURALLY INOCULATED SEED

In a microscopic study of seeds from the various lots noted in table 1, covered smut spores and an abundance of septate mycelium of a definite type commonly were found with the pericarps of the caryopses (pl. 1, *A* and *B*). In several instances the characteristic mycelium was found emanating from covered smut spores (pl. 1, *C*). Presumably, therefore, the commonly observed mycelium was that of *Ustilago hordei*. The high percentages of smutted plants so frequently obtained from dehulled seed (table 1) also indicate that the heavy load of pericarp inoculum was that of *U. hordei*.

CONCLUSIONS BASED ON STUDIES OF NATURALLY INOCULATED SEED

The foregoing results failed to substantiate the long-held belief that spores on the surface of seed constitute the prime source of infection. It would appear, instead, that inoculum, in the form of spores and mycelium from germinated spores, on and in the pericarp of the caryopsis, accounts for most of the covered smut in plants from seed lots grown, handled, and stored under farm conditions. This apparently explains one of the perplexing angles of the problem previously noted (p. 788), i. e., the frequent heavy outbreaks of covered smut in crops from naturally inoculated seed on which inoculum is not macroscopically visible and the infrequent occurrence of smut in crops from seed artificially blackened with millions of spores. Inoculum lying beneath the hulls, close to the embryo and having unobstructed access to it, manifestly might be expected to be far more effective than inoculum on the outside of the hulls. In fact, it has recently been discovered that the position of inoculum on the seed is related not only to the speed and degree of seedling infection before emergence, but also to the influence of environment after emergence on the severity of both covered smut in barley and loose smut in oats (44, 45). The foregoing results probably also explain the effectiveness of the spore-suspension method of inoculating seed barley with covered smut (40). According to this method, spores are washed beneath the hulls and the inoculated seed then is stored in a moistened condition for

24 hours to permit the spores to germinate. The method thus simulates the effective natural method of initiating infection through inoculation of the caryopsis. It is noteworthy that Jensen (23), Tisdale (47), Faris (12), Briggs (6), Johnston (25), and Aamodt and Johnston (1) likewise have reported good infection through inoculation of the caryopsis by dusting with spores after the hulls had been removed. The practical difficulties in removing the hulls, however, have prevented a wider use of this effective method.

DIFFERENT TYPES OF EMERGENCE OF COVERED SMUT HEADS AND THEIR RELATION TO SPORE DISSEMINATION AND SEED INOCULATION

In view of the foregoing results, observations were made on the type of emergence of smutted heads, and experiments were undertaken to determine, under field conditions at Arlington farm, how soon after heading the dissemination of spores and inoculation of seed begins, how long it continues, and its relative importance in different periods of seed development and in threshing. Also, the influence of different conditions during storage on the germination of seed-borne spores and on the effectiveness of different seed treatments throughout the various phases of the host-pathogen association were studied.

In regard to spore dissemination and seed inoculation in nature, a factor should be noted that may have a material influence on these processes. Apparently it has not been previously reported that there are different types of covered smut heads in respect to emergence. For example, when Odessa (C. I.⁴ 934) spring barley was grown in a greenhouse from seed inoculated with the writer's physiologic race 4 (43), the smutted heads failed to reach the auricles of the flag leaf. They emerged laterally and usually remained partly sheathed as shown in figure 1, A. However, when Odessa was similarly grown from seed inoculated with physiologic race 1, the smutted heads emerged completely above the boot (fig. 1, C). In addition to the race of smut, the variety of barley and the growing conditions apparently also may be concerned. All of the plants shown in figure 2, for example, were grown from seed similarly inoculated with spores from a single purified collection (collection 13, race 6). The winter barleys Alaska (C. I. 4106) and Nobarb (C. I. 6120) produced exserted smut heads, but the spring variety White Smyrna (C. I. 910) produced the low, semienclosed type. These three barleys were grown in adjacent rows under winter conditions at Arlington farm. When the Alaska plants were grown under greenhouse conditions, however, they produced semienclosed smut heads.

In the following studies on dissemination of inoculum and inoculation of seed, the exserted type of covered smut head was principally concerned. Based on observations to date, this seems to be the prevailing type in the standard winter barleys grown in the field at Arlington farm. It would seem likely that the greater exposure of exserted heads to wind and rain and to contact with the awns and barbs of normal heads would result in an earlier and more complete dissemination of spores before threshing than would the protected position of

⁴ C. I. refers to accession number of Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

smutted heads that are more or less enclosed. Therefore the local prevailing type of both smutted heads and weather conditions may be concerned in various degrees in the time and manner of spore dis-



FIGURE 1.—Influence of different physiologic races of covered smut on type of smut heads in Odessa barley. A, Low, semienclosed smut heads; seed inoculated with spores of race 4. B, Normal head. C, High, exerted smut heads; seed inoculated with spores of race 1.

semination and seed inoculation. This is a possibility that doubtless should be considered in applying the present results to the wide variety of conditions under which barley is grown.



For explanatory legend, see opposite page

In the light of the foregoing data, it is of interest to note that, in the literature also, the descriptions of covered smut heads frequently indicate that different types were being described, at least with respect to persistence of the smut-enclosing membrane and dissemination of spores. Frequently it is stated that the spores of barley covered smut are mostly or entirely confined to the heads by membranes that persist until the crop is threshed (8, 10, 19, 20, 22, 27, 34, 35, 37). But Güssow and Conners (16) note that covered smut in the field "may be mistaken for loose smut, which it resembles at times rather closely." Likewise, according to Clinton (9), the general appearance of loose and covered smuts—

is such as to lead one to suppose them to be the same, since they both occur as dusty outbreaks which more or less completely destroy the flower parts; [however, the covered smut] has these outbreaks protected by a membrane which thus more permanently holds the spores together.

Kellerman and Swingle (26) note that—

the covered barley smut differs from all the other loose smuts in that the attacked panicle is not at once converted into a powdery mass by the escape of the smut, but the smut remains more or less completely enclosed by a membrane [that] keeps the smut intact for some time and finally allows it to escape through rents and fissures in the membrane.

DISSEMINATION OF COVERED SMUT IN WINTER BARLEYS AT ARLINGTON FARM

The writer's observations on covered smut in the standard varieties of winter barley grown in the field at the Arlington Experiment Farm confirm those of Kellerman and Swingle noted above. A few days after the smutted heads emerge, the membranes of the sori begin to develop rents and fissures. The spore masses thus exposed become dry and powdery, and spores may be readily blown, washed, or otherwise carried to healthy heads (fig. 3). Moreover, as the plants and normal heads become dry and brittle with maturity, the smutted heads likewise undergo a process of ripening and become dry and spongy. Further splitting of peridia and dispersal of spores accompanies this process (fig. 4). The disintegration of sori and the dissemination of spores also are promoted by the moving contact of smutted and healthy heads as they wave in the breezes during the period from heading to cutting. The barbs of rough-awn barleys are especially effective in shredding the spore-enclosing membranes. Disintegration of covered smut heads in a plot of ripened, standing Wisconsin Winter barley at Arlington farm in 1936 is shown in figure 5, A. It is evident that much of the covered smut was disseminated before this grain was cut. In cutting, binding, and shocking the grain, a further dissemination of spores doubtless occurred. Finally, conditions in the shock that accompany curing and drying, at least at Arlington farm, increase further the fragility of peridia and powderiness of the smut. A representative sample of covered smut heads

Explanatory legend for figure 2

FIGURE 2.—Influence of environment (A and B) and of different varieties (B, C, and D) on type of covered smut heads. A, Alaska winter barley grown in a greenhouse; smut heads low. B, Alaska grown in the field, smut heads high. C, White Smyrna spring barley grown in the field; smut heads low. D, Nobarb winter barley grown in the field; smut heads high. A, B, C, and D were grown from seed similarly inoculated with spores from a single purified collection of covered smut; B, C, and D were grown in adjacent rows under winter conditions.

taken from a shock of Wisconsin Winter barley at Arlington farm shortly before threshing in July 1935 is shown in figure 5, *B*. It is



FIGURE 3.—*A*, Healthy head; *B*, two covered smut heads; *C*, loose smut head of Wisconsin Winter barley, showing comparative extent of disintegration in covered smut and loose smut heads toward the close of the bloom period of healthy heads in May 1936 at Arlington farm. The enclosing membranes of the covered smut heads already had broken at that time.

apparent that under the conditions that prevailed, much of the smut-head disintegration, spore dissemination, and seed inoculation has preceded threshing.

In view of the fact that some of the covered smut heads resembled those infected with loose smut, it should be noted that especial care was taken to make sure that only covered smut was present. The crop from which these smutted heads were collected had been grown from seed treated with formaldehyde solution. After treatment, the seed was divided into 2 parts. One part was dusted with spores of *Ustilago hordei*; the other was not inoculated. Both parts were sown in the fall of 1934. The uninoculated seed produced a smut-free crop. The plants from inoculated seed produced 13 percent of covered smut heads. From this smutted crop, the 6 smut heads shown in figure 5,

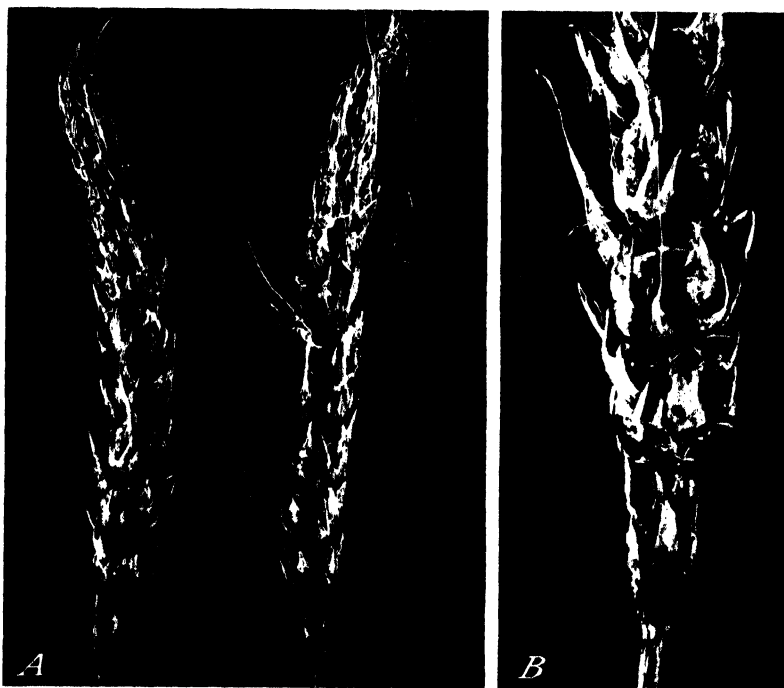


FIGURE 4.—*A*, Covered smut heads of Han River barley, showing the progress of disintegration 2 weeks after emergence in May 1935, at Arlington farm; *B*, an enlargement of the lower portion of the left head in *A*, showing the rents and fissures in the smut-enclosing membranes, which permit the release and spread of countless numbers of spores to the developing seed of nearby healthy heads.

B, were collected. Spores from each head were microscopically examined and were germinated on 2-percent potato-dextrose agar. The spores were smooth-walled and on germination produced promycelia bearing typically 4 lateral sporidia, both characters typical of *U. hordei*. In November 1935, seed of Odessa barley that had been treated with a 1 : 320 formaldehyde solution for 2 hours at 20° C. was divided into 7 parts. One part was not inoculated; each of the others was inoculated with an aqueous suspension of spores from 1 of the smut heads. Sown in a greenhouse, the uninoculated seed produced a smut-free crop, while each of the inoculated lots produced 20 to 25

plants with 90 to 100 percent of smutted heads, all of which showed the external typical characters of covered smut. Furthermore, examination of the spores from each of the 6 lots showed that their walls were smooth, and on germinating they produced the typical pro-

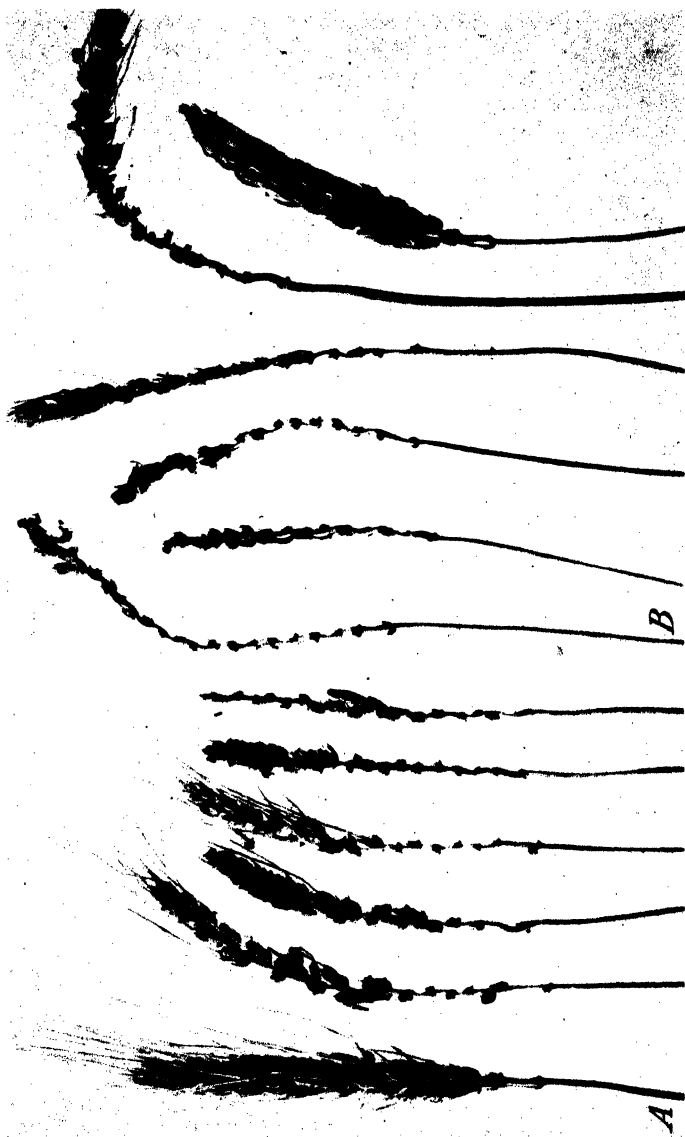


FIGURE 5.—Representative samples showing the progress of disintegration in covered smut heads of Wisconsin Winter barley at Arlington farm: A, Heads collected from standing plants a day before the grain was cut in June 1936; B, heads collected from a shock a few days before threshing in July 1935.

mycelium with lateral sporidia. There can be no doubt, therefore, that the heads shown in figure 5, B, were infected with true covered smut.

In view of these data, it seems evident that, at least under the weather conditions and with the exerted type of covered smut heads

at Arlington farm, a considerable portion of covered smut spores may be disseminated not only before the barley is threshed but even before the grain is cut.

EFFECTS OF PERIODS OF SPORE DISSEMINATION AND METHODS OF SEED STORAGE

In the light of the foregoing observations, a practical question arises as to how the severity of covered smut in the next crop is affected by spore dissemination in different periods of development of the seed barley and of different methods of seed storage.

Simultaneously with the studies on spore dissemination, experiments were conducted to determine the effectiveness of inoculum distributed to the seed (1) while still in the heads of standing plants; (2) as a result of the cutting, binding, and storage of grain in the shock; and (3) as a result of threshing. The influence of some conditions under which inoculated seed may be stored after threshing was also studied. Through this procedure it was possible to trace, step by step, the inception and early development of the host-parasite relation and to determine the importance of each step in the ultimate development of covered smut.

In a preliminary study, covered smut heads (fig. 4) were collected at Arlington farm in May 1935 from field-grown Han River winter barley. A few days later, spores sifted from the smutted heads were applied dry, by means of a small brush, to healthy heads of Cusado (C. I. 895) and Han River (C. I. 2163) barleys growing in an isolated smut-free plot. The heads thus inoculated had emerged about 2 weeks previously. At maturity in June, the inoculated heads were placed in a desiccator over calcium chloride in order to dry thoroughly and to inhibit any further development of the inoculum. After being stored in this manner until the following November, the heads were threshed by hand. After threshing, the seed was immediately returned to the desiccator except that a portion from each variety was soaked in a 1:320 formaldehyde solution at 20° C. for 1 hour, then washed in water and spread in a thin layer to dry. After 3 days this seed was sown in a greenhouse, together with the remaining seed from the desiccator. A part of the latter was treated with copper carbonate dust (50 percent copper) immediately before sowing, while another part was sown without treatment. The results are presented in table 2.

TABLE 2.—Covered smut resulting from brushing dry spores on Cusado and Han River barley heads 2 weeks after their emergence at Arlington farm, and the smut-controlling effects of copper carbonate dust and formaldehyde solution when applied to seed from the inoculated heads

Seed treatment	Heads of Cusado		Heads of Han River	
	Total	Smutted	Total	Smutted
	Number	Percent	Number	Percent
None.....	123	82.9	120	86.7
Copper carbonate dust.....	119	73.1	131	48.9
Formaldehyde solution.....	118	18.6	119	7.6

The data in table 2 show clearly that covered smut spores reaching immature barley seed in the heads of standing plants may result in

very high infection. Smut was only moderately reduced through treating the seed with copper carbonate and was not completely controlled, even after treating the seed for 1 hour with the formaldehyde solution. In view of these facts there can hardly be any doubt that some of the inoculum, probably in the form of mycelium from germinated spores, got beneath the hulls, where it was protected against the lethal action of the disinfectants.

In a further study of spore dissemination and seed inoculation, healthy heads of Wisconsin Winter barley were collected in July 1935 from a shock immediately before it was threshed. From the same shock, the smut heads shown in figure 5 *B*, also were collected. As noted previously (p. 796) only one smut was present in the crop and it was definitely identified as *Ustilago hordei*. Immediately after their collection, the healthy heads were placed in a desiccator over calcium chloride in order to dry the seed thoroughly and to inhibit any further development of inoculum that may have reached it. The following day a portion of the threshed seed also was placed in a desiccator immediately after threshing, and another portion, consisting of 2 bushels of threshed seed, was put in a grain sack and stored in a laboratory. After being stored in this manner until the following November, each lot was divided into three portions and, as in the previous test, one was treated with copper carbonate dust (50 percent copper) and one with formaldehyde solution, while the third was left untreated. These various lots were then sown in a greenhouse. The results are presented in table 3.

TABLE 3.—Relative importance in 1935 of spores distributed to seed before and during threshing on the occurrence of covered smut in Wisconsin Winter barley, and influence of 2 methods of storing threshed inoculated seed and of treating seed (with copper carbonate dust or formaldehyde solution) on the loss from smut

Source of seed	Method of storing seed, July to November	Method of treating seed, following storage	Heads	
			Total	Smutted
			Number	Percent
Shock	Stored in desiccator over calcium chloride in laboratory.	Untreated.....	75	70.7
		Copper carbonate.....	70	35.7
		Formaldehyde solution.....	79	2.5
Threshing machine	do.....	Untreated.....	70	81.4
		Copper carbonate.....	72	51.4
		Formaldehyde solution.....	70	1.4
Do.....	Stored in 2-bushel bag in laboratory.	Untreated.....	77	90.9
		Copper carbonate.....	73	39.7
		Formaldehyde solution.....	74	2.7

It will be noted in table 3 that (1) the naturally distributed inoculum on the seed before threshing resulted in a high percentage of covered smut; (2) the additional inoculum added to the seed in threshing effected a further increase of smut; and (3) the storage of threshed inoculated seed in bulk in a bag instead of storage in a desiccator resulted in an additional increase of smut. As in the preceding experiment, copper carbonate treatment of the seed proved ineffective in control, and even the 1-hour seed treatment with formaldehyde solution failed to give complete control.

In view of the definite indications of the preceding experiments on spore dissemination, the study was expanded in 1936-37 to include the complete range over which the pathogen is associated with the seed, i. e., from heading through seed development, harvest, threshing, and storage to the time of seeding. Seed of two varieties, Han River and Wisconsin Winter, was treated with formaldehyde solution, inoculated with *Ustilago hordei* by the spore-suspension method (40), and sown in $\frac{1}{80}$ -acre plots at Arlington farm in the fall of 1936. The following spring the Han River and Wisconsin Winter plots developed 26.2 and 33.4 percent of covered smut heads, respectively. Normal heads in these plots were collected at random from standing plants the day before each crop was cut. Later, heads were collected from shocks the day before the barley was threshed. In both cases the heads, immediately after collection, were placed in desiccators in the laboratory. Following threshing, a few hundred threshed seeds were immediately placed in a desiccator. Also 2 bushels of threshed seed was placed in a grain sack and stored in the laboratory near the seed in the desiccators. Seed of the various lots was stored as indicated from June to October 1936 and then divided in two portions, one of which was untreated and the other treated with copper carbonate dust (50 percent copper). Immediately thereafter, the seed was uniformly sown in special containers in which soil conditions highly favorable to infection were maintained. When the seedlings were 1 to 1½ inches above the soil they were transplanted to an outdoor plot. The results are presented in table 4.

The data in table 4 give a vivid picture of how the covered smut fungus progressively enhanced its relation with the host throughout the development, ripening, curing, threshing, and storage of the barley seed. The naturally distributed inoculum on the seed before cutting resulted in an average of 19.5 percent of smutted heads in the two varieties. The inoculum added to the seed as a result of cutting and shocking the grain raised the average to 27.1 percent. Inoculum added to the seed through final disintegration of the smutted heads in threshing brought the average to 50.6 percent. Finally, storage of the seed in bulk under prevailing atmospheric conditions evidently furnished the moisture that led to a further increase in the load of inoculum through germination of the seed-borne spores and spread of mycelium, with the result that an average of 71.3 percent of the heads were smutted. The marked increase in smut from storage of seed in a bag, as compared with that stored under very dry conditions in a desiccator, confirms the evidence of the previous year (table 3) that conditions of atmospheric humidity under which the seed is stored may have an appreciable influence on the development of inoculum borne by the seed and on the severity of smut infection. The ineffectiveness of the copper carbonate seed treatment throughout the test would seem to indicate that in these seed lots, as in the naturally inoculated lots from other parts of the country that were studied earlier (table 1), much of the effective inoculum in the form of spores or mycelium from germinated spores had passed beneath the hulls, where it was protected from the disinfectant.

In view of the fact that seed barley in the United States is harvested and stored under a wide range of moisture conditions, the influence of different atmospheric humidities during seed storage on severity and control of covered smut was studied further. The lot of barley used was a hulled variety (Norway, C. 1. 2535), definitely known to harbor no naturally deposited smut inoculum. The seed was artificially blackened with spores and each of 6 lots (200 seeds per lot) was placed in a small wire basket suspended midway within a 1-pint mason jar. No water was added to the first jar, and 5, 10, 15, 20, and 25 drops of water were placed in the second to sixth jars, respectively. The water was deposited within each jar by means of a pipette so that none of the fluid touched the suspended seed. Immediately thereafter each jar was tightly sealed and stored in a 20° C. chamber for 1 month. The seed was then removed from the jars, and each of the 6 lots was divided into 2 equal parts, 1 of which was left untreated, the other treated with copper carbonate dust (50 percent copper). The seed then was sown in a greenhouse. The results (table 5) show clearly that different degrees of atmospheric moisture to which inoculated seed is exposed in storage may markedly influence the incidence of covered smut in the succeeding crop and also the effectiveness of a seed disinfectant. Gage (15) has reported similar results with oat smut. The fact that copper carbonate failed to control the smut on seed stored under more humid conditions would seem to indicate that under those conditions the spores germinated in storage and developed a mycelium that grew beneath the hulls, where it was protected from the disinfectant. In the bottles with 20 and 25 drops of water, the increasingly heavy growth of mold that developed on the seed in storage probably interfered with development of the smut and doubtless accounts for the lower percentages of infection.

TABLE 5.—Incidence of covered smut in Norway barley as influenced by storing seed superficially blackened with spores under different atmospheric humidities for 1 month at 20° C., and the influence of the different humidities during storage on the effectiveness of the copper carbonate seed treatment applied immediately after storage

Drops of water in storage jar	Plants from seed given indicated treatment after storage				Mold on seed at conclusion of storage period
	None		Copper carbonate		
	Total plants	Smutted plants	Total plants	Smutted plants	
	<i>Number</i>	<i>Percent</i>	<i>Number</i>	<i>Percent</i>	
0.....	92	80.9	100	0	No visible mold.
5.....	84	69.0	84	0	Do.
10.....	72	75.0	96	0	Do.
15.....	80	87.5	100	28.0	Slight growth of mold.
20.....	92	82.6	98	18.4	Moderate growth of mold.
25.....	76	36.8	92	8.7	Heavy growth of mold.

In connection with these results it may be noted that seed treatment with copper carbonate dust has not been effective enough to warrant general recommendation. However, in California, according to Mackie (32), thorough and repeated dusting of the seed with copper carbonate every year appears to eliminate covered smut satisfactorily.

Under dry conditions of harvest and storage in California, or elsewhere, spores that reach the seed possibly may not germinate and the inoculum therefore may remain largely confined to the seed surface, where it would be susceptible to control with copper carbonate.

In view of the foregoing findings on spore dissemination and seed inoculation, it is evident that, from the time the smutted heads emerge to the time the seed is sown, environmental factors and the conditions of handling and storing the smutted seed play an important role in the incidence of covered smut in the succeeding crop. Through their influence on the dissemination of spores and on the germination of spores after reaching the seed, environmental factors and handling and storage conditions are importantly related to the load of inoculum carried by the seed, the position of the inoculum in relation to the embryo, and the effectiveness of seed treatments for control. When it is further considered that the incidence of covered smut in barley may be markedly influenced by the interaction of various soil conditions from seeding to emergence (11), by environmental conditions after the seedlings have emerged (44, 45), and by the various degrees of varietal susceptibility to the various physiologic races of the covered smut fungus (12, 43), the multiplicity of interacting environmental and biologic factors concerned in the ultimate incidence of this smut in barley becomes apparent.

DISCUSSION

As already noted (p. 787), the inoculation of seed barley with covered smut has been difficult to understand. Naturally inoculated seed frequently has produced heavily smutted crops, while seed artificially blackened with millions of spores usually has produced crops with a surprisingly low incidence of smut. The factors underlying Faris' (11) exceptional success with the spore-dusting method likewise have not been understood. With the facts brought to light in the present study and with the aid of other data recently found on the nature of infection (44, 45), it seems possible now to explain these discrepancies.

The present study has clearly shown that, in the successful natural method of seed inoculation, much of the effective inoculum becomes established beneath the hulls. In the ineffective artificial method, however, the inoculum is deposited on the exterior of the hulls. It would seem reasonable that inoculum residing on the caryopsis and having immediate and unobstructed access to the embryo should be able to invade the seedling more rapidly and effectively during its subterranean growth than inoculum elsewhere on the hulls. The different results from the natural and artificial methods of inoculation thus might be ascribed to the position of the inoculum on the seed relative to the hulls. As already noted (p. 788), however, certain results reported by Faris (11) definitely appear to challenge this hypothesis. By applying spores to the surface of Hannchen seed barley with the hulls intact, Faris obtained up to 97.8 percent of smutted plants. This would appear to be substantial evidence that the hulls were not effective barriers to infection. However, in the light of a recent discovery, it now appears that the hulls probably always interfere with infection but that their deterrent influence may be masked by a compensating factor. It has been found, recently, that the incidence of covered smut in barley and of loose smut in oats may be markedly

influenced by certain environmental conditions after the seedlings emerge (44, 45). For example, the writer (40), inoculated seed by the superficial dusting method and also by the spore-suspension method, in which the inoculum is deposited beneath the seed hulls as with naturally inoculated seed. After the seed had germinated under controlled soil conditions favorable to infection, seedlings were transplanted in autumn or winter to a field or greenhouse. Field and greenhouse plants from seed inoculated by the spore-suspension method produced similar high percentages of smut.

When seed was inoculated by the superficial dusting method, plants grown in a greenhouse from emergence to maturity also produced a high percentage of smut but plants grown in the field after emergence produced only a low or appreciably lower percentage of smut. The superficial dusting method of inoculation therefore appeared effective when the plants were grown in a temperate environment after seedling emergence, and less effective or ineffective when the plants were grown after emergence under the colder, more rugged conditions to which winter grains are exposed in the field at Arlington farm. In the experiment of Faris with Hannchen spring barley noted above, success with the superficial dusting method of inoculation likewise was linked with a temperate post-emergence environment. In contrast, the investigators reporting unsatisfactory results with the dusting method of seed inoculation grew their plants under field conditions (1, 3, 6, 23, 25, 32, 36, 47). It is noteworthy also that, in an experiment with two winter barleys, Faris (12) obtained no smut whatsoever when spore-dusted seed was germinated under soil conditions favorable to infection and the plants were grown in the field from seedling emergence in October to maturity the following summer. However, when the plants were grown in a greenhouse at 55° to 70° F. after emergence, considerable smut developed.

These results therefore seem to warrant the following conclusions in explanation of the difficulties in understanding the inoculation and infection of barley by the covered smut: In general, and within limits, the incidence of covered smut in plants from naturally inoculated seed frequently is high because inoculum finds its way beneath the hulls under natural conditions of inoculation. Through the advantage of proximity to the embryo, the pathogen is facilitated in establishing a deep-seated infection from seeding to seedling emergence. For infection of the seedling, therefore, the fungus becomes less dependent upon the uncertainty of favorable climatic conditions after emergence. When inoculum resides on the surface of seed, as happens when seed is artificially dusted with spores, it is evidently more difficult for the pathogen quickly to establish a deep-seated preemergence infection, owing to the obstructing hulls. Success with this method, then, depends more upon the hazard of a period of temperate climatic conditions after the seedlings emerge. In this account, it is assumed, of course, that the soil conditions from seeding to seedling emergence have been at least somewhat favorable for infection.

From the results of previous studies (11, 44, 45) and those of the present investigation, the host-parasite relation of barley and the covered smut fungus now may be traced from its inception at heading, through the ripening, harvesting, threshing, and storage of the seed, and from seeding through seedling emergence to maturity (11, 44, 45).

This information should serve a practical purpose in breeding for resistance to covered smut. It has already served a useful purpose in the development of the spore-suspension method of seed inoculation (40), a new artificial method patterned after the natural method, which is practicable and has been highly and consistently effective under field and greenhouse conditions of barley culture.

SUMMARY AND CONCLUSIONS

A practicable and effective artificial method of inoculating seed barley with covered smut has been diligently sought for many years. No satisfactory method being available, it has been difficult to determine adequately the smut resistance of barley varieties and to breed for resistance against the heavy annual toll of covered smut. Years of general observation have convincingly established the fact that inoculation of seed as it occurs in the usual field culture of barley is effective and frequently results in high percentages of smut. The object of the present study, therefore, was to determine the factor or factors responsible for the effectiveness of the natural method and to use the knowledge in developing a successful artificial method.

It has long been generally considered that, under the natural or field conditions of inoculation, spores of barley covered smut are held in the smutted heads until threshing and that inoculation comes about when the smutted heads are disintegrated in threshing and free spores stick to the surface of the seed hulls of barley. The artificial blackening of seed with millions of spores, however, repeatedly has failed to result in good infections. Furthermore, the treatment of naturally inoculated seed with certain surface disinfectants usually has failed to result in satisfactory control, indicating that the effective inoculum is beneath rather than upon the seed hulls.

Through a study of naturally inoculated seed lots from Eastern, Central, and Western States it was found that the most effective inoculum consisted not of spores upon the seed hulls but of spores and of extensive ramifications of mycelium from germinated spores on the pericarps of the caryopses beneath the seed hulls. The occurrence of this subhull inoculum in nature evidently explains why copper carbonate dust, formaldehyde solution, and some other surface disinfectants of seed barley repeatedly have been reported as being relatively ineffective in the control of covered smut throughout most of the barley-growing areas of the United States.

In the light of another discovery on the covered smut of barley that has been recently reported, it seems possible now to explain also why the subhull inoculum of naturally inoculated seed has been generally effective and the superficial inoculum of artificially inoculated seed generally ineffective. It has been shown that when seed is sown and the inoculum is beneath the hulls, the pathogen is frequently able to establish such a deep-seated infection of the seedling before it emerges that exposure to cold after emergence has relatively little adverse influence on infection. When the inoculum resides on the exterior of the seed, however, the nature of the infection is such that exposure to cold after the seedlings emerge usually promotes a lowered incidence of smut. High infections may occur, but they are dependent on the hazard of a moderate climatic period of several weeks or more following emergence.

In a study of spore dissemination and seed inoculation in the field at Arlington Experiment Farm, Arlington, Va., it was found that all of the spores of barley covered smut are not held intact in the smutted heads until threshing, as has been generally reported. A few days after the smutted heads emerge, the membranes that enclose the spores begin to split, thus permitting an early dissemination of spores and an early inoculation of developing seed in healthy heads. Disintegration of smutted heads, dissemination of spores, and inoculation of seed continue throughout the development, maturation, curing, and drying of the standing and shocked grain, and culminate with the completion of threshing.

All of the spores that reach the seed do not lie dormant on the surface of hulls until seeding. Some that are blown, washed, or otherwise carried to the seed from heading to threshing time may come to lie beneath the hulls or send infection hyphae beneath the hulls or both. Likewise, spores that reach the seed during threshing also may send infection hyphae beneath the hulls under certain conditions of moisture during storage. Thus, beginning a few days after the smutted heads emerge and continuing to the time when the seed is sown, the pathogen may pursue its course in the subhull region of the seed.

The relative importance of dissemination of inoculum and seed inoculation during different periods of seed development and under different conditions of seed storage at Arlington farm was ascertained in 1935 and 1936. The results of both years were definite and similar in their indications that, from heading through ripening, cutting, shocking, threshing, and storage, the covered smut fungus progressively improved both its entrenchment in the subhull region of the seed and its relation to infection of the seedling upon seed germination.

In a further study of storage conditions, progressive increases in the atmospheric humidities in which inoculated seed was stored resulted in progressive increases in the incidence of covered smut, up to the point when the growth of mold on the seed became appreciable. Also, the copper carbonate dust treatment ceased to be effective in control when applied to seed stored at the higher humidities. The seed was artificially inoculated through blackening the surface with spores.

Two different types of emergence of covered smut heads are described and illustrated. In the one type, the smutted heads fail to reach the auricles of the flag leaf and remain half enclosed in the boot. In the other type, the smutted heads become fully exerted. The latter type chiefly occurs in the standard winter barleys at Arlington farm, and it was this type of covered smut that was used in the present study. Whether spores are disseminated more rapidly from the exerted than from the semienclosed type of covered smut head has not been determined. Intermediate and irregular types also have been observed. Preliminary studies seem to indicate that the variety of barley, the physiologic race of smut, and the conditions under which the plants are grown are factors concerned in the different types of emergence of the smutted head.

The recently extended knowledge of factors that condition the infection of barley by *Ustilago hordei* has aided in the development of the spore-suspension method of seed inoculation. This is a new artificial method, patterned after the natural method, that has proved to be highly and consistently effective as well as practicable.

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FOLIAR DIAGNOSIS OF DIFFERENTIALLY FERTILIZED GREENHOUSE TOMATOES WITH AND WITHOUT MANURE¹

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INTRODUCTION

The method of foliar diagnosis (4)² has demonstrated that deductions drawn solely from observations of the relations between the fertilizer applied and the yields are very restricted and may not infrequently be erroneous (5, 6).

Such errors result from lack of knowledge of the processes of nutrition which have determined the yields. To illustrate: A fertilizer element may be absorbed and utilized by the plant and yet its application may result in a reduction in yields through its effect on the absorption of another element (7, 8). In the present experiment, the yields alone would have given no indication that the higher yields of the manured over the unmanured plots taken as a group were due to the higher level of potash nutrition produced by the manure.

The method of foliar diagnosis has not, thus far, been applied to plants of the indeterminate type of growth, such as tomatoes under glass. Certain statements, frequently met with in the literature, to the effect that artificial conditions in a greenhouse result in obscuring or obliterating the specific effects of mineral nutrients on leaf development would possibly lead to the conclusion that the method of foliar diagnosis fails to show any relationship between the mineral nutrition and the development of the plant. But, as the results reported herein show, such a conclusion would be erroneous. The fallacy lies in the failure to recognize the fundamental principle inherent in the method of foliar diagnosis, namely, that in the examination of the nutrition of morphologically homologous leaves sampled from plants at the same time, the values are used in a comparative manner only (2).

MATERIAL AND METHODS

DESCRIPTION OF THE BEDS

A detailed description of the beds and also of the method of culture of the plants has been given by Mack (1). Each bed is divided into 2 plots of equal size, 5 $\frac{3}{4}$ feet wide by 8 feet long, with 12 plants in 3 rows of 4 plants each, lengthwise of the plot. The surface soil is a composted clay loam, 14 inches in depth, with a clay loam subsoil. The plants in the experiment reported in this paper were selected for uniformity and were grown from seed of a Pennsylvania certified strain of the Marglobe variety of tomatoes (*Lycopersicum esculentum* Mill.).

Each pair of plots in a bed received the same fertilizer treatment, but one of them received in addition an annual application of well-rotted horse manure just before the fall crop was planted.

¹ Received for publication October 30, 1939. Paper No. 929 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

² Italic numbers in parentheses refer to Literature Cited, p. 832.

THE FERTILIZER APPLIED

The fertilizer treatments consisted of a single element, combinations of two elements, and also of all three elements at the rate of 260.8 gm. of sodium nitrate, 625.9 gm. of superphosphate, and 95.2 gm. of muriate of potash per plot, equivalent to 41.73 gm. of N, 125.19 gm. of P_2O_5 , and 43.1 gm. of K_2O . The single applications were equivalent to 500 pounds of nitrate of soda, 1,250 pounds of superphosphate, and 350 pounds of muriate of potash to the acre. Manure was applied at the rate of 110 pounds per plot, equivalent to 50 tons to the acre. This quantity (110 pounds) contained 424.1 gm. of N, 279.4 gm. of P_2O_5 , and 344.3 gm. of K_2O . No *in vitro* tests of indisputable validity are known for determining the availability of the fertilizer elements in manure. In the present experiment the availability of the elements could be gauged from the results on the plot which received manure only.

The different treatments are shown in table 1 in which N, P, and K designate the amounts of the respective carriers, (2N) twice the amount, and (RN) biweekly applications of the standard amount of nitrogen throughout the growing period; this was approximately equal to 2,086.4 gm. of nitrogen. The letter m indicates manure. The plants were watered when necessary by allowing water to run from a hose for the same length of time on each plot. Plants were set in the beds on March 9. They were trained to upright single stems, and the stem was cut off at a height of about 7 feet. Fruits were harvested when well-colored.

LEAF SAMPLING

Effective sampling from such a relatively small number of plants, 12 in each plot, presented difficulties, especially when individual differences among plants of a plot were quite marked in certain plots. In sampling plants grown under field conditions (4, 5, 6, 7, 8, 9), leaf samples were not taken from those plants that deviated considerably from the average of the plot. This practice, if adopted in the greenhouse, would have given too small a sample; nor could the customary method of sampling plants lengthwise across a plot be adopted, for by carrying out such a system only 4 plants would have been available in each plot. The compromise adopted was to sample the fifth leaf from the base on all plants on a plot at 3 sampling dates, April 5, April 29, and May 17, as follows:

At the first sampling two or three leaflets were taken on one side of the midrib of the leaf; at the second sampling, the leaflets opposite those removed at the first were selected; and at the third sampling, the remaining lateral leaflets and the terminal leaflet were taken as a sample.

Owing to senescence of the fifth leaf on many of the poorly nourished plants, a fourth sampling was not possible 3 weeks after the third samples were taken. In plants of indeterminate type of growth, as in this experiment, it would have been possible to have extended the observations by using the method of relay sampling described in an earlier paper (4).

ANALYTICAL METHODS

The preparation of samples and the analytical methods used were identical with those described earlier (4) and were such as to give the total nitrogen, the total phosphoric acid, and the total potash present

in the leaf at the moment of sampling, irrespective of the form in which each was present.

PRESENTATION OF RESULTS

The plots with their treatments and yields are given in table 1.

The percentages of nitrogen, phosphoric acid, and potash in the fifth leaf from the base, at each date of sampling are shown in table 2, together with the intensities of nutrition and the composition of the NPK units. These data are shown graphically in the accompanying figures.

TABLE 1.—*Plot treatments and yields of fruit*

Plot No.	Treatment	N, P ₂ O ₅ , and K ₂ O equivalent applied to each plot	Symbol	Yield of fruit
		Grams		Pounds
10R	Nothing		O	88.1
10L	Manure		m	113.2
12L	Sodium nitrate	41.73	N	64.4
12R	Sodium nitrate+manure		N+m	121.9
14L	Superphosphate	125.19	P	43.3
14R	Superphosphate+manure		P+m	113.5
16L	Sodium nitrate	41.73	} NP	30.9
	Superphosphate	125.19		
16R	Sodium nitrate } Superphosphate } + manure		NP+m	117.9
18L	Sodium nitrate	41.73	} NK	95.6
	Potassium chloride	43.10		
18R	Sodium nitrate } Potassium chloride } + manure		NK+m	120.5
20L	Superphosphate	125.19	} PK	98.8
	Potassium chloride	43.10		
20R	Superphosphate } Potassium chloride } + manure		PK+m	129.3
2R	Sodium nitrate	125.19	} NPK	113.8
	Superphosphate	43.10		
2L	Sodium nitrate } Potassium chloride } + manure		NPK+m	121.8
4R	Sodium nitrate	83.46	} (2N)PK	107.3
	Superphosphate	125.19		
4L	Sodium nitrate } Potassium chloride } + manure	43.10	(2N)PK+m	115.5
8R	Sodium nitrate	333.84	} (RN)PK	104.5
	Superphosphate	125.19		
8L	Sodium nitrate } Superphosphate } + manure	43.10	(RN)PK+m	120.5
	Potassium chloride			

TABLE 2.—The percentages of N, P₂O₅, and K₂O in the fifth leaf, their milligram-equivalent values, and the composition of the NPK-units at the three dates of sampling, for plants grown under different fertilizer treatments

Date	Mineral content of dried foliage				Milligram-equivalent			Composition of NPK-units			
	N	P ₂ O ₅	K ₂ O	N+P ₂ O ₅ +K ₂ O	N	P ₂ O ₅	K ₂ O	S	X	Y	Z
	(M _N)	(M _P)	(M _K)	(M _S)	(E _N)	(E _P)	(E _K)	(E _S +E _K +E _X)	$\left(100 \times \frac{E_X}{S}\right)$	$\left(100 \times \frac{E_Y}{S}\right)$	$\left(100 \times \frac{E_Z}{S}\right)$

NOTHING (PLOT NO. 10R)											
April 5.....	percent	percent	percent	percent	292.744	79.143	31.843	393.730	71.811	20.101	8.087
April 29.....	3.990	1.871	1.465	7.326	207.060	105.750	13.759	327.485	63.227	32.291	4.481
May 27.....	2.140	3.066	.646	5.852	152.796	129.691		296.246	51.577	43.778	4.044

MANURE (PLOT NO. 10L)											
April 5.....	percent	percent	percent	percent	292.744	56.836	77.319	416.900	67.804	13.653	18.542
April 29.....	2.880	1.346	3.630	8.936	205.632	73.856	40.597	320.985	64.243	23.074	12.683
May 27.....	2.140	1.833	2.067	6.040	152.796	77.535	44.027	274.358	55.692	28.260	16.047

N (PLOT NO. 12L)											
April 5.....	4.420	1.190	1.455	7.035	315.588	49.914	30.991	396.493	79.592	12.589	7.817
April 29.....	3.140	1.690	.835	5.575	224.196	67.680	17.785	308.561	72.404	21.856	5.740
May 27.....	2.520	1.840	.671	5.031	179.928	77.832	14.292	272.052	66.137	28.608	5.253

N+MANURE (PLOT NO. 12R)											
April 5.....	4.690	1.080	3.049	8.789	332.724	45.684	64.944	443.352	75.047	10.304	14.648
April 29.....	3.700	1.840	2.454	7.654	284.180	63.450	52.270	379.900	69.339	16.701	13.758
May 27.....	2.900	1.646	1.925	6.471	207.060	69.626	41.602	317.638	65.177	21.916	12.906

P (PLOT NO. 14L)											
April 5.....	3.900	1.866	0.852	6.638	278.400	79.778	18.147	376.385	73.983	21.106	4.821
April 29.....	2.380	2.386	.667	5.433	169.932	100.025	14.207	285.067	59.611	35.405	4.963
May 27.....	1.940	2.826	.646	5.312	131.376	119.540	13.790	264.676	49.614	45.183	5.202

P+MANURE (PLOT NO. 14R)

April 5.....	3.680	1.013	3.490	8.183	262.752	42.830	74.337	379.939	69.156	11.278	19.566
April 29.....	2.600	1.673	1.698	5.971	185.640	70.788	36.167	292.575	63.450	24.187	12.361
May 27.....	2.180	1.746	1.647	5.573	155.352	73.856	35.081	264.589	58.827	27.913	13.288

NP (PLOT NO. 16L)

April 5.....	4.440	1.473	1.137	7.050	317.016	62.308	24.218	403.542	78.559	15.438	6.001
April 29.....	2.740	1.920	1.665	5.325	202.776	81.216	14.164	298.156	68.010	27.239	4.750
May 27.....	2.000	2.393	1.594	4.987	142.800	101.224	12.652	256.676	55.634	39.436	4.929

NP+MANURE (PLOT NO. 16R)

April 5.....	4.520	1.200	3.727	9.147	322.728	50.760	79.385	452.873	71.261	11.208	17.529
April 29.....	3.240	1.646	1.925	6.811	231.336	69.626	41.002	341.954	67.651	20.360	11.989
May 27.....	2.480	1.690	1.744	5.824	177.072	67.680	37.147	281.899	62.813	24.008	13.177

NK (PLOT NO. 18L)

April 5.....	4.520	1.113	2.067	7.700	322.728	47.080	44.027	413.835	77.984	11.376	10.638
April 29.....	3.080	1.453	1.901	5.194	219.912	47.080	21.321	288.313	76.275	16.329	7.395
May 27.....	2.420	1.313	1.775	4.508	172.788	55.540	16.507	244.835	70.573	22.084	6.741

NK+MANURE (PLOT NO. 18R)

April 5.....	4.660	1.313	2.096	8.169	332.724	55.539	46.774	435.037	76.481	12.776	10.751
April 29.....	3.640	1.453	2.648	7.741	259.886	61.462	56.402	377.760	68.799	16.270	11.989
May 27.....	3.080	2.173	2.648	7.901	219.912	91.918	56.402	308.252	59.720	24.962	15.317

PK (PLOT NO. 20L)

April 5.....	3.680	1.393	2.069	7.172	262.752	58.924	44.708	366.384	71.715	16.081	12.202
April 29.....	2.500	1.773	1.292	5.265	157.080	73.000	27.518	259.598	60.509	28.860	10.698
May 27.....	1.700	1.990	1.033	4.693	121.380	82.908	22.903	226.291	53.638	36.637	9.723

Date	Mineral content of dried foliage				Milligram-equivalent				Composition of NPK-units			
	N (M _F)	P ₂ O ₅ (M _F)	K ₂ O (M _F)	N+P ₂ O ₅ +K ₂ O (M _F)	N (E _F)	P ₂ O ₅ (E _F)	K ₂ O (E _F)	S (E _F +E _P +E _K)	X' $\left(100 \times \frac{E_{F'}}{S}\right)$	Y' $\left(100 \times \frac{E_{P'}}{S}\right)$	Z' $\left(100 \times \frac{E_{K'}}{S}\right)$	
PK+MANURE (Plot No. 20R)												
April 5.....	4.260	1.300	4.166	9.726	304.164	54.990	88.736	447.890	67.910	12.277	19.812	
April 29.....	3.020	1.686	2.680	7.386	215.628	71.319	57.084	344.031	62.676	20.730	16.592	
May 27.....	2.300	1.920	2.196	6.416	164.420	81.216	46.774	292.210	56.199	27.793	16.006	
NPK (PLOT NO. 2R)												
April 5.....	4.360	1.220	1.753	7.373	311.304	52.029	37.978	401.311	77.571	12.964	9.463	
April 29.....	3.180	1.560	1.076	5.816	227.052	65.968	22.919	315.959	71.861	20.885	7.253	
May 27.....	2.400	1.893	.826	5.119	171.360	80.074	17.364	269.028	63.695	29.764	6.539	
NPK+MANURE (PLOT NO. 2L)												
April 5.....	4.360	1.184	3.727	9.271	311.304	50.083	79.385	440.772	70.627	11.363	18.010	
April 29.....	3.200	.946	2.874	7.020	228.480	40.116	61.216	329.712	69.296	12.136	13.566	
May 27.....	2.760	1.366	2.209	6.335	197.064	57.782	47.051	301.897	65.275	19.140	15.585	
(2N) PK (PLOT NO. 4R)												
April 5.....	4.600	1.213	1.873	7.686	328.440	51.309	39.895	419.644	78.266	12.226	9.507	
April 29.....	3.320	1.600	.961	5.901	237.044	67.060	20.895	325.023	72.797	20.785	6.417	
May 27.....	2.720	2.133	.923	5.798	194.208	91.072	19.702	304.982	63.678	26.961	6.460	
(2N) PK+MANURE (PLOT NO. 4L)												
April 5.....	4.540	1.240	2.842	8.622	324.156	52.452	60.534	437.142	74.153	11.998	13.847	
April 29.....	3.460	1.480	2.704	7.644	247.044	62.044	37.595	367.243	67.269	17.046	15.684	
May 27.....	2.800	1.366	1.593	5.749	199.920	58.628	33.292	291.840	98.503	20.080	11.407	

(RN) PK (PLOT NO. 8R)

April 5.....	4.820	1.226	2.164	8.210	344.148	51.859	46.083	442.100	77.843	11.730	10.425
April 20.....	3.880	1.320	1.306	6.509	277.032	55.896	27.881	360.749	76.793	15.478	7.728
May 27.....	3.090	1.640	.861	5.581	219.912	69.372	18.339	397.623	71.487	22.551	5.961

(RN) PK+MANURE (PLOT NO. 8L)

April 5.....	4.620	1.233	3.681	8.934	329.868	51.156	65.625	446.649	73.853	11.453	14.692
April 20.....	3.790	1.280	2.667	7.727	299.862	54.144	56.907	380.843	70.866	14.216	14.916
May 27.....	3.220	1.590	2.357	6.837	229.908	53.298	50.204	333.410	68.956	15.965	15.057

DISCUSSION OF RESULTS

THE YIELDS FROM THE RESPECTIVE TREATMENTS

The order of yields in the unmanured series is not the same as that of the manured series. In the unmanured series the order in descending values is $NPK > (2N) PK > (RN) PK > PK > NK > \text{nothing} > N > P > NP$; and in the manured series $PK + m > N + m > NPK + m > \left\{ \begin{array}{l} (RN) PK + m \\ \text{and} \\ NK + m \end{array} \right\} > NP + m > (2N) PK + m > \left\{ \begin{array}{l} \text{manure} \\ \text{and} \\ P + m \end{array} \right\}$. In the unman-

ured series the four lowest yielding plots were those that received no potash. In the manured series the manure obscured, in general, the effects of an element added in the commercial fertilizer. Each element, however, increased the yields over the plot which received manure only. The highest yield resulted from the addition of potash and phosphate only, to manure. Increased quantities of nitrate in a complete fertilizer resulted in a reduction of yields in both the manured and unmanured plots.

THE GRAPHS SHOWING THE PERCENTAGES OF N, P_2O_5 , AND K_2O AS ORDINATES AND DATES OF SAMPLING AS ABSCISSAE

PLOTS WITHOUT MANURE

The nitrogen graphs (fig. 1) show that the nitrogen decreased progressively with increasing age of the leaves in all cases. At the first date of sampling, the nitrogen content of the leaves was higher in plants which received nitrate additions than in those that did not. The higher position of the graphs of the former indicates that this holds true for the whole cycle except in NP (plot No. 16L) during the latter part of the cycle.

The graphs for nitrogen are the highest and in increasing order in the leaves of plants which received twice the unit amount and biweekly unit additions of nitrogen, respectively. The fact that yields are in descending order the higher the graph, indicates that at the prevailing levels of phosphoric acid and potash, the buffer capacity of the plants for nitrogen has been exceeded in (RN) PK (plot No. 8R) and also in (2N) PK (plot No. 4R). But, beyond this, no relationship exists between the position of the nitrogen graphs and the yields from the various treatments.

The course of the graphs for phosphoric acid is abnormal, inasmuch as the graphs all slope upward with increasing age of the leaves, indicating progressively greater supply relative to demand of phosphoric acid with increasing maturity. The slope is least steep in the plot receiving biweekly additions of nitrogen (plot No. 8R) which shows that greater utilization of phosphoric acid has occurred. These facts suggest that nitrogen and phosphoric acid are out of physiological balance (3). The relationship is best considered from the viewpoint of the equilibrium between $N-P_2O_5-K_2O$ (pp. 823-826).

That this composted soil is very rich in phosphoric acid is indicated by the high content of the leaves of the check (nothing) plot (No. 10R) even at the first date of sampling. As a result, no relationship is apparent between the phosphoric acid content of the leaf and its presence in the fertilizer. Because of the greater accumulation of phosphoric acid with increasing age of the leaf in the lowest yielding plots, an inverse relationship of the phosphoric acid content of the leaves to yields exists. Moreover, except when accompanied

by nitrogen and potash, no relationship exists between the presence of phosphoric acid in the fertilizers and the yield.

The graphs for potash are abnormally low from all unmanured treatments. They are far below the nitrogen graphs throughout the cycle and even below those of phosphoric acid during the latter portion. The addition of potash is, however, reflected in the higher positions of the graphs of plants which received muriate. Moreover, the two lowest graphs for potash, NP (plot No. 16L) and P (plot No. 14L) are associated with the lowest yielding plots, and are fol-

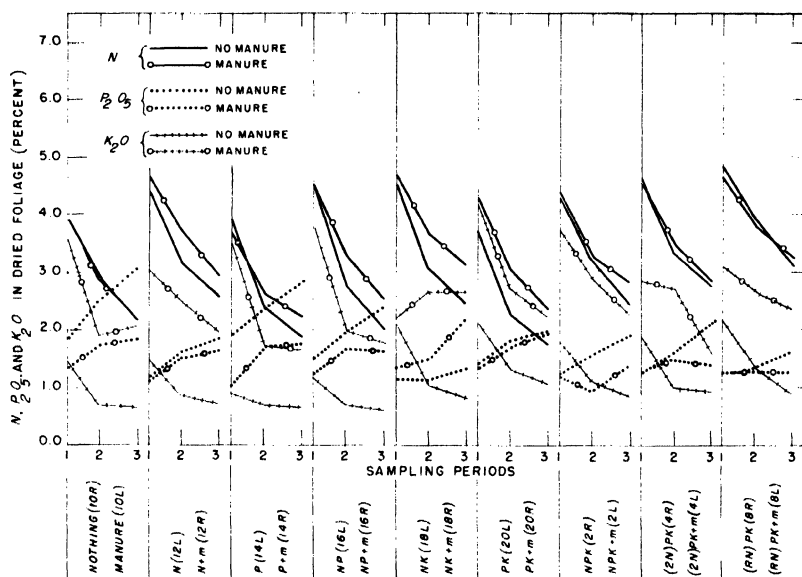


FIGURE 1.—Changes during the growth cycle in the content of N, P_2O_5 , and K_2O in the fifth leaf resulting from the different treatments indicated with plot numbers in parentheses. Percentage values in terms of the dried foliage are given as ordinates, and dates of sampling as abscissae. The three dates of sampling (April 5, April 27, and May 29) are indicated by the digits 1, 2, and 3, respectively, along the base line.

lowed in order of yields and percentage of potash by the next two lowest yielding plots N (plot No. 12L) and check (plot No. 10R). In the unmanured plots there is, therefore, a relationship between the potash in the leaf and its presence in the fertilizer, between potash in the leaf and the yields, and between the presence of potash in the fertilizer and the yield.

THE MANURED PLOTS

The influence of nitrate additions is reflected also in the higher positions of the nitrogen graphs of the leaves from plants growing on the nitrated plots throughout the cycle. But no relationship exists between the height of a graph and the yield.

As in the unmanured series, no relationship exists between the phosphoric acid content of the leaf and its presence in the manure and mineral fertilizer.

At the first date of sampling, the potash content of the leaf is

highest in the highest yielding plot PK+m (plot No. 20R). But with this exception and contrary to the findings in the unmanured plots at this date, the influence of the mineral potash additions on the potash content of the leaf is masked by the influence of the potash in the manure. Thus, the K_2O content of the leaf in NP+m (plot No. 16R) is as high as that of NPK+m (plot No. 21L), and that of the check (nothing) is much higher than those of (2N) PK+m (plot No. 4L) and of (RN) PK+m (plot No. 8L). But with increasing maturity of the leaf, the influence of potash in the muriate is felt for in general, the graphs of the manured plots containing added mineral potash are less steep than of those of the unmanured plots and as a result, with one exception, end higher.

COMPARISON OF THE GRAPHS OF THE MANURED AND UNMANURED SERIES

The nitrogen graph of plot 10L which received dressings of manure only coincides with that of the check plot, No. 10R. Furthermore, the nitrogen graphs of the manured complete-fertilizer plots (Nos. 4L and 8L) differ little from their respective unmanured plots (Nos. 4R and 8R), and the differences are not very great in P (plot Nos. 14L and 14R) except during the latter part of the cycle. However, relatively wide differences exist between the yields of the manured and the corresponding unmanured plot. In these treatments differences in the nitrogen content are not the factor causing differences in yields between the manured and its particular unmanured companion plot.

Greater differences between the graph of the manured and the corresponding unmanured plot are observed in N (plot Nos. 12L and 12R), NP (plot Nos. 16L and 16R), NK (plot Nos. 18L and 18R), and PK (plot Nos. 20L and 20R), in which the graph of the higher yielding manured plot is higher than that of the corresponding unmanured plot. In these plots, then, yields can be associated with differences in nitrogen content of the leaf.

With one exception, the graphs of phosphoric acid in the manured plots are below those of the corresponding unmanured plots. The exception is NK+m (plot No. 18R). The fact that manure dressings alone (plot No. 10L) have caused a reduction in the phosphoric acid content of the leaf much below that of the check or nothing plot (No. 10R), even at the first date of sampling, indicates that the seat of this inhibition lies in the soil, presumably, as the result of adsorption. The fact that little influence of the manure is observed in PK+m (plot No. 20R) throughout the cycle, and that the effect of the manure is small in the complete fertilizer plots at the early period, and is absent from NK+m (plot No. 18R) suggests that the forces causing the adsorption have been reduced by the addition of muriate of potash. The reduction in the phosphoric acid is associated with higher yields in the manured plots. But there is no evidence that differences in the content of phosphoric acid of the leaves of plants growing in the manured and unmanured plots, respectively, are a causal factor in producing the differences in yields. For example, wide differences in yields exist between a manured and its corresponding unmanured plot even when their graphs for phosphoric acid lie close together throughout the cycle.

The position of the lowest potash graphs for the manured plots is higher than that of the highest potash graphs for the unmanured

plots throughout the whole cycle, and, furthermore, the graph for potash of each manured plot is much higher than that of the unmanured plot throughout the whole cycle. The differences, moreover, are very much greater than those between the nitrogen graphs or the phosphoric acid graphs of any manured and unmanured pair. These facts suggest that with sufficiently high levels of nitrogen and phosphoric acid, potash is the determining factor that has produced these differences in yields between the manured and unmanured series. This relationship is best shown in the equilibrium between nitrogen, phosphoric acid, and potash considered later.

INTENSITIES OF NUTRITION AND EQUILIBRIUM BETWEEN NITROGEN, PHOSPHORIC ACID, AND POTASH AT SUCCESSIVE DATES OF SAMPLING

The graphs of figure 1 just discussed show in terms of the percentages of the dried foliage the relationship of supply of a particular element to demand by the plant for that element with increasing age of the leaf. They do not readily show, however, their physiological relationships to one another.

To show these relationships fully the percentages must be converted into milligram-equivalent values, arranged in such form as will indicate the equilibrium between nitrogen, phosphoric acid, and potash at each date of sampling. This has been done in the manner already described in earlier papers (4, 6, 7). In table 2, the values for X , Y , and Z in the last three columns represent the milligram-equivalent values for nitrogen, phosphoric acid, and potash, respectively, expressed as a combined NPK unit (4). This unit is derived by finding the proportion which each of the milligram-equivalent values shown in columns 10, 11, and 12 bears to the milligram-equivalent total. The values thus obtained are multiplied by 100 in order to avoid fractional magnitudes.

The analytical data obtained on samples collected on April 5 from the nothing plot (No. 10R) may be used to illustrate the method of calculating the NPK-unit.

$$\text{Percent N} = M_x = 3.960; \text{ percent } P_2O_5 = M_y = 1.871; \text{ percent } K_2O = M_z = 1.495.$$

$$\text{Then } s = M_x + M_y + M_z = 7.326, \text{ and}$$

$$E_x = 1,000 \frac{M_x}{N} = 71.4 \times 3.960 = 282.744$$

$$E_y = 1,000 \frac{M_y}{1/6(P_2O_5)} = 42.3 \times 1.871 = 79.143$$

$$E_z = 1,000 \frac{M_z}{1/2(K_2O)} = 21.3 \times 1.495 = 31.843$$

$$E_x + E_y + E_z = S = 393.730$$

$$X = 100 \times \frac{E_x}{S} = \frac{100 \times 282.744}{393.730} = 71.811$$

$$Y = 100 \times \frac{E_y}{S} = \frac{100 \times 79.143}{393.730} = 20.101$$

$$Z = 100 \times \frac{E_z}{S} = \frac{100 \times 31.843}{393.730} = 8.087$$

The values 71.811, 20.101, and 8.087 represent the values of the NPK unit of the fifth leaf taken April 5 from the check (nothing) plot.

The intensity of nutrition (s) consists of the sum of the percentages of nitrogen, phosphoric acid, and potash ($N+P_2O_5+K_2O$) at the moment of sampling a leaf and is shown in the fifth column of table 2.

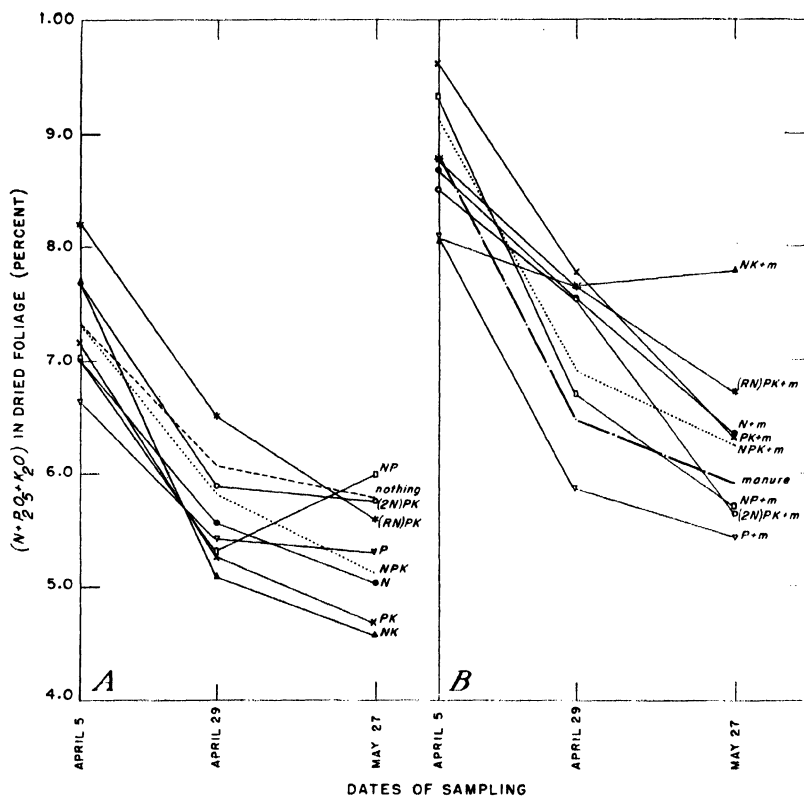


FIGURE 2.—Intensities of nutrition of the leaves at the three dates of sampling. Percentage values of ($N+P_2O_5+K_2O$) in the dried foliage as ordinates and dates of sampling as abscissae: A, not manured; B, manured.

The method of foliar diagnosis has demonstrated that a fertilizer can be used intelligently only if it is selected with a knowledge of the manner in which it will affect the intensity, or the composition of the NPK-unit, or both simultaneously. The values for the intensities resulting from the various treatments with increasing age of the leaf are shown in figure 2, and the values for the physiological relations—the NPK-units—in trilinear coordinates in figures 3 to 6, inclusive.

THE INTENSITIES OF NUTRITION

In all treatments in the manured as well as in the unmanured series, except NK+m, Plot No. 18R, where a slight increase occurs in the last period, the intensities of nutrition decreased with increasing age of the leaf, in spite of the fact that one constituent (phosphoric acid)

of these magnitudes increased with increasing maturity of the leaf in all cases. This abnormal behavior of phosphoric acid did, however, modify the length and steepness of the slopes of figure 2 with increasing age of the leaf. This influence was especially noticeable in the unmanured plots. At the first date of sampling the lowest values for the intensities were associated with plots having the lowest yields, namely, P (plot No. 14L), NP (plot No. 16L), and N (plot No. 12L), and the highest value but one for the intensity with the treatment giving the highest yield, namely, PK+m (plot No. 20R). Furthermore, the intensity of the higher yielding manured plot was always higher than that of its unmanured companion plot. The intensity alone is not, however, the deciding factor in determining yields. It has been established by foliar diagnosis (5, 7) that a low intensity of nutrition cannot compensate for a relatively good equilibrium between the elements, although a high intensity may compensate for a relatively poor equilibrium (5, 7). So too, in the present experiment, the relatively low intensity of nutrition of the plot receiving manure only (plot No. 10L) resulted in the lowest yields of the manured plots, although the NPK equilibrium was relatively good.

THE EQUILIBRIUM BETWEEN NITROGEN, PHOSPHORIC ACID, AND POTASH

GENERAL CHARACTERISTICS OF THE GRAPHS

The influence of the increase of phosphoric acid with increasing maturity of the leaves from all treatments, to which reference has already been made (p. 818), although affecting the intensities of nutrition but little, is reflected in the composition of the NPK-units. In figures 3 to 6 this is shown by the great displacements towards the right base apex representing $P_2O_5=100$ percent with increasing maturity of the leaf, and is especially noticeable in the unmanured plots. To conserve space in figures 3 to 6 only a part of the triangle, each side of which equals 100, is shown. On the other hand, the graphs of manured and unmanured plots alike, with increasing maturity, are displaced away from the summit of the triangle representing $N=100$ percent, indicating that the values for nitrogen in the NPK-unit decrease progressively with increasing age of the leaf. With two exceptions, the graphs for potash are displaced away from the apex representing $K_2O=100$ percent, indicating a decrease in the potash in the unit with increasing age of the leaf. The graph of each manured plot is displaced towards the left base apex in comparison with the graph of its unmanured companion plot, so that not only is the percentage of potash in the leaf of the manured plot higher (figure 1) but also its proportion relative to nitrogen and phosphoric acid in the NPK-unit is higher than that of the unmanured plot: The difference in the values for K_2O in the NPK-unit between any manured and unmanured pair is not less than 200 percent at any time.

Except in one case of manured and unmanured plots the graph of each manured plot is shorter than that of the unmanured one. And contrary to the relative positions of the nitrogen graphs of a manured and the corresponding unmanured plot in figure 1, based on the percentages of nitrogen in the dried material of the leaf, the trilinear coordinate graph of a manured plot is always displaced lower in the triangle at the first date of sampling than that of the unmanured one, indicating that in the former the proportion of nitrogen relative to

phosphoric acid and potash is less than in the latter. With increasing age of the leaf the displacement of the graph of a manured plot relative to that of the unmanured plot becomes less and less and in

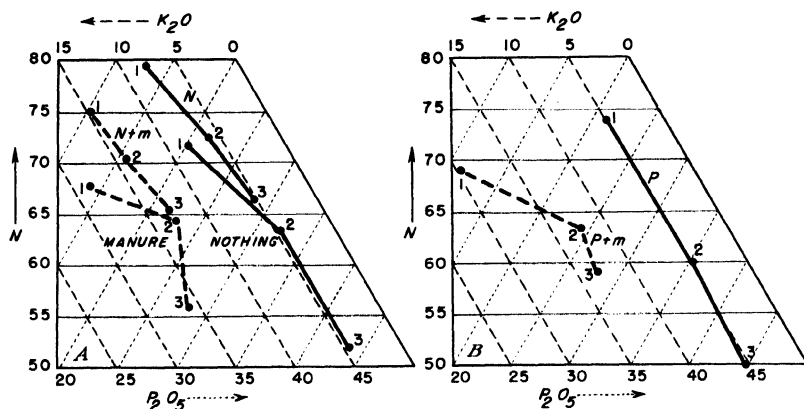


FIGURE 3.—Changes during the growth cycle in the N-P₂O₅-K₂O equilibrium in the fifth leaf from plants growing on: A, Plots No. 10R (nothing), No. 10L (manure), No. 12L (N), and No. 12R (N + manure); B, plots No. 14L (P) and No. 14R (P + manure). The composition of the NPK unit at the successive dates of sampling, April 5, April 27, and May 29, is indicated by the numerals 1, 2, 3, respectively.

most pairs is reversed during the later portion of the growth cycle. Accordingly, with increasing maturity the relative proportion of nitrogen in the NPK-unit is higher in the manured than in the unmanured

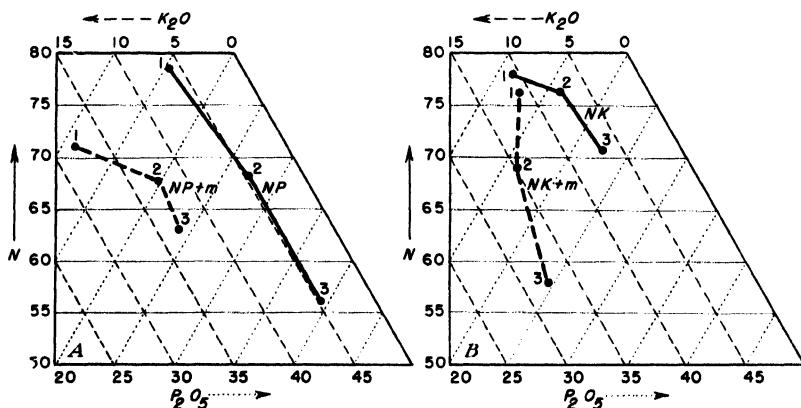


FIGURE 4.—Changes during the growth cycle in the N-P₂O₅-K₂O equilibrium in the fifth leaf from plants growing on: A, Plots No. 16L (NP) and No. 16R (NP + manure); B, plots No. 18L (NK) and 18R (NK + manure). The composition of the NPK unit at the successive dates of sampling, April 5, April 27, and May 29, is indicated by the numerals 1, 2, 3, respectively.

plot. Since in figure 1, little difference occurs between the forms and positions of the nitrogen graphs of the nothing and manure plots (Nos. 10R and 10L) and between (2N) PK and (RN) PK with their respective manured plots, the only possible conclusion is that the

fertilizer additions have made the nitrogen in the manure more available. This increased availability is masked in plots 4R and 4L and also in 8R and 8L by the increased additions of mineral nitrogen.

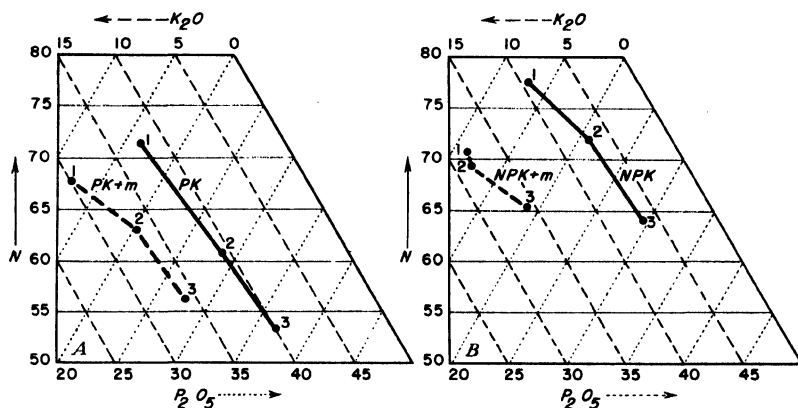


FIGURE 5.—Changes during the growth cycle in the N-P₂O₅-K₂O equilibrium in the fifth leaf from plants growing on: A, Plots No. 20L (PK) and 20R (PK + manure); B, plots No. 21R (NPK) and 21L (NPK + manure). The composition of the NPK unit at the successive dates of sampling, April 5, April 27, and May 29, is indicated by the numerals 1, 2, 3, respectively.

Except in one case, NK and the manured plot corresponding (plots 18L and 18R, fig. 6) the graph of each manured plot is displaced, relative to that of the corresponding unmanured one, away from the

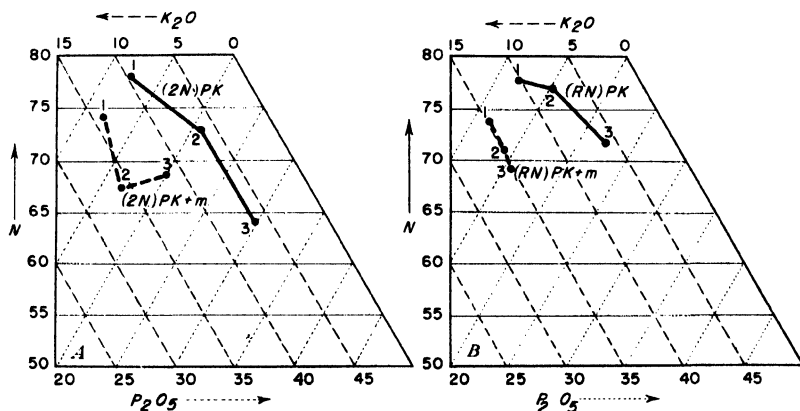


FIGURE 6.—Changes during the growth cycle in the N-P₂O₅-K₂O equilibrium in the fifth leaf from plants growing on: A, Plots No. 4R ((2N)PK) and 4L ((2N)PK + manure); B, plots No. 8R ((RN)PK) and 8L ((RN)PK + manure). The composition of the NPK unit at the successive dates of sampling, April 5, April 27, and May 29, is indicated by the numerals 1, 2, 3, respectively.

right base apex throughout the cycle, indicating that the proportion of phosphoric acid in the NPK-unit has been reduced. In the NPK-units, then, the relationship between phosphoric acid of the manured and unmanured plots is not changed from that indicated in figure 1.

THE COURSE OF THE TRILINEAR COORDINATE GRAPHS DURING THE GROWTH CYCLE

In the plots 10R (*nothing*) and 10L (*manure*), the relatively long and steep slope away from the summit ($N=100$ percent and towards the right base apex ($P_2O_5=100$ percent) of the check plot (No. 10R) indicates the rapid increase in phosphoric acid with increasing age of the leaf. This increase is made at the expense of the nitrogen and also of the potash (fig. 3, A).

The graph of manure (plot No. 10L) is shorter and is less steep than that of the nothing (plot No. 10R) and is displaced relative to it away from the right base apex ($P_2O_5=100$ percent) and towards the left base apex ($K_2O=100$ percent).

As compared with the graphs of plots 10R and of 10L, respectively, the graphs of the unmanured and manured plots Nos. 12L (N) and 12R (N+manure) (fig. 3, A), both of which received nitrate additions, are displaced toward the summit of the triangle and further away from the right base apex. This displacement indicates that the addition of nitrate of soda has resulted in an increase in the relative proportions of nitrogen, a reduction in the phosphoric acid, and not much change in the respective values for potash. Relative to the unmanured plot, the graph of the manured plot is displaced towards the left base apex, further away from the right base apex, and away from the summit of the triangle.

The forms of the graphs of the manured and unmanured plots Nos. 14L (P) and 14R (P+manure) (fig. 3, B), also Nos. 16L (NP) and 16R (NP+manure) (fig. 4, A), are very similar. The graphs of the unmanured plots are characterized by having the longest and steepest slopes towards the right base apex and away from the summit of any of the graphs, indicating that these treatments which have given the lowest yields are associated with the greatest reduction of nitrogen in the NPK-unit and with the greatest increase in phosphoric acid during the cycle, but with little change in the already very low values for potash.

The addition of nitrogen to phosphate is reflected in the displacement of the graph of 16L (fig. 4, A) towards the summit. The addition of manure has led to a reduction in the lengths of the graphs and in displacements toward the left base apex and away from the right base apex. At the first date of sampling, the graphs of the manured plots are displaced lower relative to the respective unmanured plots, but with increasing age of the leaf the relative displacements become less and less and are finally reversed in the latter part of the growth cycle.

Plots 18L (NK) and 18R (NK+manure) (fig. 4, B) are the only pair in which the graph of the unmanured plot is shorter than that of the corresponding manured one, indicating the exception to the findings in the other pairs that dressings of manure have reduced the relative proportion of phosphoric acid in the NPK-unit. In this pair the graph of the manured plot is below that of the unmanured during the whole cycle and not merely during the early portion.

As in other plots without nitrate additions the absence of this element is reflected in the positions and elongation of the graphs in plot Nos. 20L (PK) and 20R (PK+manure) (fig. 5, A). The graph of plot No. 20L is characterized by being nearer to the left base apex ($K_2O=100$ percent) than any of the manured plots during the whole cycle.

The similarity in lengths and positions of the graphs of the respective manured and unmanured plots Nos. 2R (NPK) and 2L (NPK+manure) (fig. 5, *B*); Nos. 4R ((2N)PK) and 4L ((2N) PK+manure) (fig. 6, *A*); and Nos. 8R ((RN) PK) and 8L ((RN) PK+manure) (fig. 6, *B*) is an indication that the addition of twice the unit amounts of nitrate of soda did not affect appreciably the proportions of nitrogen or of phosphoric acid or of potash in the NPK-units. The graph of plot 8R relative to those of plots 2R and of 4R is shorter and is displaced higher in the triangle, indicating that biweekly additions of the

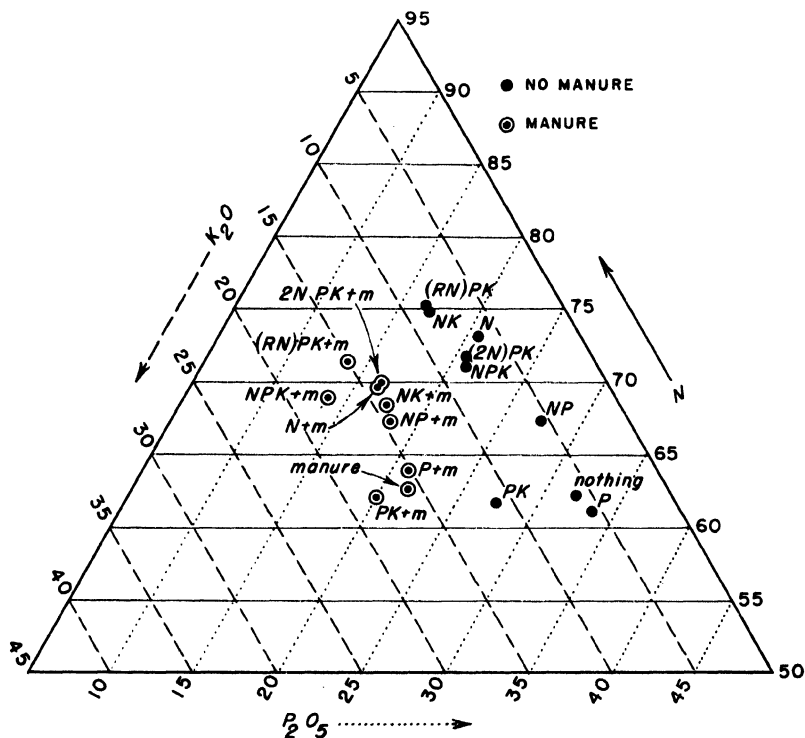


FIGURE 7.— Relative positions of the mean NPK-units of the fifth leaf from plants growing on the plots indicated. The mean NPK-unit represents the center of gravity of the respective graphs of figures 3 to 6, inclusive.

unit quantity of nitrate of soda has resulted in an increase in the proportion of nitrogen in the NPK-unit especially during the later stages of growth, a reduction in the phosphoric acid, and not much change in potash. The graphs of the respective manured plots are displaced towards the left base apex, away from the right base apex, and are lower in plot 8L throughout the cycle and in plots 2L and 4L during the early period.

THE MEAN INTENSITIES OF NUTRITION AND THE MEAN NPK-UNITS IN RELATION TO YIELDS

If yields are a measure of the practical results secured by a fertilizer, then the foliar diagnosis shows, as we have seen, the course of nutrition

with respect to the dominant fertilizer elements which determine these yields.

In relating yields to the foliar diagnosis, it is simpler to consider the resultant effects on the nutrition as indicated by values for the intensities and NPK-units, respectively, which are the means of the values at the different dates of sampling. When this is done the NPK-units can be represented by one point only, of which the absolute position on the triangle and the relative position with reference to the others are indicated at a glance.

Table 3 shows the mean of the values given in table 2 for the intensities and the composition of the NPK-units, respectively, at the several dates of sampling, in the case of each fertilizer treatment. These values are shown graphically in figure 7. By keeping in mind the fact that a fertilizer intervenes in the nutrition of a plant to affect (1) the intensity of nutrition or (2) the composition of the NPK-units, or both simultaneously, the data shown in table 3 can be analyzed from the point of view of the effects of the treatments on the mean intensity, or on the mean NPK-units, or on both simultaneously, and their relationship to the resulting yields.

TABLE 3.—*The mean intensities of nutrition, the mean NPK-units, and the yields of fruit on the various plots under different fertilizer treatments*

Plot No.	Treatment	Mean intensity of nutrition	Mean composition of the NPK unit			Yield of fruit
			N	P ₂ O ₅	K ₂ O	
			Percent	Percent	Percent	Pounds
10R	Nothing	6.422	62.205	32.057	5.737	88.1
10L	Manure	7.169	62.579	21.662	15.757	113.2
12L	N	5.887	72.711	21.018	6.270	64.4
12R	N+manure	7.638	69.921	16.307	13.771	121.9
14L	P	5.794	61.069	33.928	5.002	43.3
14R	P+manure	6.576	63.811	21.126	15.065	113.5
16L	NP	5.787	67.401	27.371	5.227	30.9
16R	NP+manure	7.361	67.242	18.525	14.232	117.9
18L	NK	5.801	74.944	16.796	8.258	95.6
18R	NK+manure	7.937	68.333	17.999	13.666	120.5
20L	PK	5.710	61.954	27.199	10.844	98.8
20R	PK+manure	7.843	62.262	20.267	17.470	129.3
2R	NPK	6.103	71.042	21.204	7.752	113.8
2L	NPK+manure	7.542	68.399	14.213	17.383	121.8
4R	(2N)PK	6.462	71.580	20.957	7.461	107.3
4L	(2N)PK+manure	7.338	69.975	16.378	13.646	115.3
8R	(RN)PK	6.767	75.374	16.586	8.038	101.5
8L	(RN)PK+manure	7.833	71.225	13.885	14.888	120.5

THE UNMANURED PLOTS

As the result of the abnormal course of phosphoric acid with increasing age of the leaf no relationship exists between the mean values for the intensities and the yields of the unmanured plots. A relationship exists, however, between the mean values of the respective NPK-units and yields. Thus, the two lowest yielding plots NP (plot No. 16L) and P (plot No. 14L) are displaced the farthest from the left base apex, followed in order of position by the next lowest yielding treatments N (plot No. 12L) and check (plot No. 10R). The relatively small quantity of muriate added to each plot is reflected, therefore, by an increase in the K₂O of the NPK unit in the leaves of plants growing on the plots which received potash as muriate. With respect to the maximum yielding unmanured plots NPK (plot No. 2R) and

(2N)PK (plot No. 4R), the next lower in order of yields (RN) PK (plot No. 8R) and NK (plot No. 18L), have higher nitrogen and lower phosphoric acid. The next in descending order of yield, PK (plot No. 20L), has lower nitrogen and higher phosphoric acid. Of the four lowest yielding plots with lower potash, three also have lower values for nitrogen and two have higher values for phosphoric acid.

THE MANURED PLOTS

The influence of manure in reducing the accumulation of phosphoric acid with increasing age of the leaf, to which attention has already been called (p. 820), has resulted in a closer relationship between intensities and yields than is shown in the unmanured plots. Thus in the manured plots the four lowest yielding plots, manure (plot No. 10L), P+m (plot No. 14R), NP+m (plot No. 16R), and (2N)PK+m (plot No. 41) have the lowest mean values for intensities. In these manured plots high yields are associated with intensities of 7.54 or higher, and relatively low yields with intensities of 7.36 or lower. Although relatively high intensities thus are a necessary requisite for high yields, the determining factor in causing differences in yields among high yielding plots, however, must be looked for in the differences in the composition of the NPK-units. The highest yielding plot, PK+manure (plot No. 20R) has a mean NPK-unit in round numbers of 62.2 : 20.3 : 17.5. The next highest yielding plots, NPK+manure (plot No. 21L), N+manure (plot No. 12R), NK+manure (plot No. 18R), and (RN)PK+manure (plot No. 8L) have the following composition:

12R.....	69.9	16.3	13.8	Yield 121.9 pounds.
21L.....	68.4	14.2	17.4	Yield 121.8 pounds.
18R.....	68.3	18.0	13.7	Yield 120.5 pounds.
8L.....	71.2	13.9	14.9	

In these plots with somewhat lower yields than the optimum, the values for the nitrogen of the NPK unit are much higher than the optimum. This increase in the nitrogen has been made at the expense of both the phosphoric acid and potash in the $N-P_2O_5-K_2O$ equilibrium, but principally at the expense of the latter. In the lowest yielding of the manured series having too low intensities, two, namely, P+m (plot No. 14R) and M (plot 10L), have too low potash relative to the optimum, and two, namely, (2N) PK (plot No. 4L) and NP (plot No. 16R), in addition to too low potash, have too high N and too low P_2O_5 .

COMPARISON OF THE VALUES FOR THE MEAN INTENSITIES OF NUTRITION AND MEAN NPK UNITS OF THE MANURED AND UNMANURED PLOTS

In comparing the values for the mean intensities of nutrition and mean NPK units, it is to be noted that the higher yields of the manured over the unmanured plots, whether these received mineral fertilizers or not, are associated with higher mean intensities in the former. Moreover, as indicated in figure 7, the position of the mean NPK units of all the manured plots as a group is displaced relative to that of the unmanured plots towards the left base apex, indicating higher potash. They are also less scattered over the triangle than are those of the unmanured series. These facts indicate that the higher yielding manured plots as a group have higher mean intensities and a

greater relative proportion of potash in the NPK unit than the unmanured plots taken as a group. These generalizations hold also for the relationship of the mean intensity and the mean NPK unit of a particular treatment to that of its unmanured companion plot.

With one exception, a relationship also exists between the higher yields of the manured plots and the lower relative proportion of the mean value of the phosphoric acid in each of these plots respectively, as compared with the corresponding unmanured plots. But this exception may vitiate a casual relationship to yields. Considering the intensities and positions on the triangle of the NPK units (fig. 7) of the manured and unmanured series as a whole in relation to the optimum yielding plot, PK+m (plot No. 20R), the intensities of the unmanured series are too low and the positions of all the NPK units of the unmanured series lie too far from the left base apex, indicating too low potash. But beyond these characteristics, the manured and unmanured series show no other consistent relationship to yields.

Thus no consistent relationships relative to the summit (N=100 percent) of the triangle are observed between the position of the mean NPK units of the manured and unmanured plots as a group, or between the position of a manured plot and its unmanured companion. The proportion of nitrogen in the mean NPK unit, then, shows no causal relationship to yields. In fact, the third lowest value for N, viz, 62.26 of the highest yielding plot (PK+manure), must be regarded as sufficiently high for optimum yields. Similarly, differences in the proportion of phosphoric acid in the NPK unit show no consistent relation to yields.

SUMMARY

The method of foliar diagnosis has been applied to plants of the indeterminate type of growth, namely, tomatoes (*Lycopersicum esculentum* Mill.) grown under commercial greenhouse conditions.

The fertilizer treatments consisted of a single element, combinations of two elements, and also combinations of all three elements, nitrogen, phosphoric acid, and potash, with and without dressings of manure. The quantities of phosphoric acid and of potash in the commercial fertilizer remained unchanged in all treatments, but the quantity of nitrogen was varied in two cases. In the one double the standard unit amount was used, and in the other the unit quantity of nitrogen was added at biweekly intervals. In all cases the quantities of rotted horse manure added to the manured series remained unchanged.

The fifth leaf from the base of each plant was sampled at three different periods during the growth cycle and was analyzed according to prescribed methods.

The results of the analyses are recorded graphically by two methods, one indicating the relation of supply of an element to demand of the element by the plant with increasing age of the leaf in terms of percentages of the dried material, and the other by means of two magnitudes representing (1) the intensity of nutrition at the moment of sampling a leaf and (2) the physiological relationships between the elements also at the moment of sampling. The latter is shown in trilinear coordinates by means of a value designated the NPK unit, which represents the equilibrium between N, P_2O_5 , and K_2O .

The order of yields in relation to the fertilizer treatment was not the same in the manured and unmanured series, respectively. In the

latter the highest yields resulted from the application of a complete fertilizer, while in the former from phosphate and potash without nitrate additions.

In both manured and unmanured series increased additions of nitrate of soda reduced yields below that from unit additions. The reduction in yields in both cases was associated with too high values for nitrogen, absolutely in terms of percentages in the dried foliage and also its proportion relative to phosphoric acid and potash in the composition of the NPK unit—values that exceeded the buffer capacity of the plant for this element at the prevailing levels of phosphoric acid and potash.

In the unmanured series the four lowest yielding plots were those which did not receive muriate of potash, and the foliar diagnosis showed that the leaves from the plants on the two lowest yielding plots had the lowest percentages of potash throughout the cycle. These plots were followed in order of yields and percentage of potash by the two other plots which did not receive any potash. Associated with the lowest yields were also the highest values for phosphoric acid throughout the cycle.

The relationships of foliar diagnosis to yields is most readily determined from the values representing the intensities of nutrition and the NPK units, respectively, and especially to the magnitudes representing their respective mean values.

In the unmanured series, because of far greater accumulation of phosphoric acid in some treatments than in others, a relationship existed between the intensity and the yield only at the first date of sampling. In these unmanured series, the highest yielding plots had the highest intensities of nutrition, and the lowest yielding the lowest intensities throughout the cycle.

In the unmanured series, the two lowest yielding plots were the furthest displaced from the left base apex where $K_2O=100$ percent, and were followed in position with respect to this apex by the two next lowest yielding plots.

High yields were associated with high values for the intensities of nutrition in the manured series and low yields with low values. In the manured plots having relatively high values for the intensities of nutrition but with yields lower than the optimum, the reduction in yields was associated with a much higher value for nitrogen in the NPK unit, made at the expense of the potash and also of the phosphoric acid, but principally of the former.

The lowest yielding plots in the manured series were associated not only with relatively low values for the intensities but also with too high a proportion of nitrogen in the NPK unit. In addition to too low values for nitrogen, in two cases [P+m] and [manure] the values for potash were found to be too low and those for phosphoric acid too high; and the two other low-yielding plots in the manured series [NP+m] and [(2N)PK+m] the values for phosphoric acid were too low.

The manured series of plots as a group gave higher yields than the unmanured series and each manured plot had a higher yield than the unmanured plot receiving a similar application of commercial fertilizer. Relative to these facts the foliar diagnosis shows that the increased yields of the manured over the unmanured plots as a group are associated with higher intensities of nutrition throughout the

cycle, and with a great increase in the potash of the NPK-unit, but with nitrogen and phosphoric acid showing no consistent relationships.

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EFFECT OF CERTAIN CHEMICAL ATTRIBUTES OF VEGETATION ON FOREST INFLAMMABILITY¹

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INTRODUCTION

Forest Service administrators and fire-research men have long felt the need of information concerning the effect of vegetation such as shrubs, grasses, and forbs (nongrasslike herbs) on the rate of spread of fires. To date, all knowledge of the subject has been acquired empirically in the field, or deduced from knowledge of fire behavior as influenced by the condition of deadwood, duff, etc.³ It is known that the luxuriant green vegetation of late spring and early summer does not lend itself to dangerous, fast-spreading fires, and that the matured and naturally cured or dried vegetation of September and October materially augments forest inflammability. Beyond this, the whole matter is one of conjecture. No information is available as to quantitative effects of forest undergrowth conditions on rate of fire spread, and no method has been evolved for determining readily and accurately whether these conditions at any particular time are such as would retard fire spread. Furthermore, little is known as to variation in volume of vegetation from season to season. Consequently, forest officers rating fire conditions and forecasting the approach of fire danger do not know what significance to assign to volume and condition of vegetation. Before fire danger can be properly rated on areas bearing large volumes of shrubs, grasses, and forbs, methods must be devised for evaluating these factors with reference to the spread of fire and for integrating data on these factors with measurements of the factors being utilized in fire-danger ratings at the present time—wind velocity and moisture content of dead fuels.

The problem of evaluating the effect of vegetation on rate of fire spread is fundamentally that of determining the heat-energy balance, that is, the ratio between the total heat energy available and the heat required to eliminate the moisture present, in whatever form. If the energy consumed in dissipating the plant moisture and bringing about combustion of the vegetative material is less than the energy produced by the combustion, the vegetation accelerates the spread of fire; if greater, the vegetation retards the spread.

The Northern Rocky Mountain Forest and Range Experiment Station, which has pioneered in the field of fire-danger rating, began, in 1935, studies of vegetation conditions in order to refine the methods of danger rating. The purpose of this work is to find measurable

¹ Received for publication December 16, 1939. The research reported here was conducted at the Priest River experimental forest of the Northern Rocky Mountain Forest and Range Experiment Station, U. S. Forest Service. This station's field of operations comprises a small portion of South Dakota, all of Montana, northern Idaho, and a portion of eastern Washington.

² Associate professor, Montana State University, employed during the summer months as a member of the Northern Rocky Mountain Forest and Range Experiment Station.

³ HAWLEY, L. F. THEORETICAL CONSIDERATIONS REGARDING FACTORS WHICH INFLUENCE FOREST FIRES. *Jour. Forestry* 24: 756-763. 1926.

factors that will represent vegetation conditions throughout each fire season and to incorporate measurements of these factors into the scheme now being used to rate fire danger. The project is divided into three phases: Vegetation volume and appearance studies, dealing with current and total volume growth and visible physical changes that occur in the plants; chemical investigations; and test fires, which are essential for determining actual behavior of fires as affected by vegetation volume and condition. The first two phases should evolve a theory or hypothesis susceptible of being proved or disproved by the third. The physicochemical variations of certain typical plants as revealed by the exploratory work to date are described in this report.

The plants selected as typical of vegetative fuels on open forest areas of northern Idaho are: Shrubs, snowbrush (*Ceanothus velutinus*), ninebark (*Opulaster pauciflorus*), and willows (*Salix* spp.); grasses, downy chess or "cheatgrass" (*Bromus tectorum*) and pincgrass (*Calamagrostis* sp.); forb, fireweed (*Chamaenerion angustifolium*).

At the beginning of the chemical investigation, in 1935, the plan was to examine the vegetation with respect to moisture and fuel content, giving special attention to seasonal variations of both factors. It was assumed that the available fuel content could be represented by the crude fiber plus the crude fats or ether extractives, that is, the resins, waxes, and oils. The crude fats are of special interest because of their high inflammability. The crude fiber determinations were discontinued in 1936, because they had failed completely to show any significant variations corresponding to changes in fire behavior after the plants attained their structural growth. To determine more exactly the relation between the water content and the potential heat energy of vegetation, calorific determinations were begun in 1936. Moisture content, calorific value, and crude fat content were measured throughout the 1936 and 1937 fire seasons in one locality, the environs of the Priest River experimental forest in northern Idaho.

PROCEDURES AND RESULTS

In all cases, only green or curing material was collected; thoroughly cured portions were excluded. Of the shrubs only the leaves were used, but of the other plants the entire aerial portions.

A 500-gm. sample of each species was collected every 10 days throughout the summer season. The sampling was always done at 8 a. m., to reduce the effects of diurnal variations. Samples for moisture determination were sealed in moisture-proof cans and taken directly to the laboratory for weighing. The materials to be used in the crude fat and calorific determinations were allowed to dry on wire trays in a warm, well-ventilated room, then were ground to a powder and passed through a 60-mesh sieve.

Moisture content was determined by drying the materials in an oven at 105° C. until there was no further significant loss of weight. Results for the 1937 season are given in table 1.

A Parr peroxide bomb calorimeter was used satisfactorily in the calorific determinations. The size of the particles to which the oven-dried vegetation had been reduced insured complete combustion. The chemicals were passed through an 80-mesh sieve before use. Results for the 1937 season are given in table 2.

Total crude fat was determined by extraction with ether according to methods outlined by the Association of Official Agricultural Chemists.⁴ Results for the 1937 season are given in table 3.

TABLE 1.—*Percent of moisture¹ in vegetation of open forest areas as determined at 105° C. in 1937*

Species ²	June 20	June 30	July 10	July 20	July 30	Aug. 10	Aug. 20	Aug. 30	Sept. 10
Snowbrush	215	212	191	177	165	146	131	120	112
Ninebark	197	218	206	148	150	137	115	101	109
Willows	230	235	183	162	156	137	123	120	113
Downy chess ³	150	116	24	9					
Pinegrass	182	176	160	152	138	129	118	107	110
Fireweed	426	341	305	290	294	257	244	249	241

¹ All values were calculated on the basis of oven-dry weight of single samples, checked by 2 or more similar determinations made in the course of other phases of this project.

² The snowbrush sample was composed of leaves of various ages in proportion to those on the plant. For ninebark and willows, leaves of the current year only were collected. For downy chess, pinegrass, and fireweed, the entire aerial portion (current year's growth) was used.

³ On July 30 and later no green growth was present.

TABLE 2.—*Calories per gram, on basis of oven-dry weight,¹ determined on vegetation of open forest areas in 1937*

Species	June 20	June 30	July 10	July 20	July 30	Aug. 10	Aug. 20	Aug. 30	Sept. 10	Average
Snowbrush	4,600	4,800	4,900	5,000	5,000	5,000	5,100	5,200	5,200	4,990
Ninebark	5,000	4,500	4,600	4,600	4,700	4,800	4,700	4,600	4,700	4,690
Willows	4,600	4,800	4,800	4,800	4,700	4,700	4,700	4,700	4,800	4,730
Downy chess ³	4,300	4,300	4,400	4,300						4,330
Pinegrass	4,200	4,000	4,100	3,900	3,900	4,100	4,000	3,900	3,800	3,990
Fireweed	4,300	4,300	4,300	4,400	4,400	4,400	4,400	4,400	4,400	4,360

¹ All determinations were computed to an accuracy of 1 calorie per gram, but for the sake of comparison the figures are rounded off to the nearest hundred. Each tabulated value is the average of 2 determinations agreeing within 30 calories.

² Probably affected by a sampling error.

³ On July 30 and later, no green growth was present.

TABLE 3.—*Percent of crude fat¹ in vegetation of open forest areas, 1937*

Species	June 20	June 30	July 10	July 20	July 30	Aug. 10	Aug. 20	Aug. 30	Sept. 10
Snowbrush	5.3	7.2	7.0	7.6	9.2	12.8	11.2	10.5	11.8
Ninebark	7.2	3.7	5.7	5.5	6.6	7.1	6.5	6.4	7.3
Willows	5.7	6.4	6.1	6.0	6.6	6.6	6.7	6.0	6.2
Downy chess ³	2.5	2.6	3.3	2.2					
Pinegrass	4.4	3.4	4.1	3.6	4.0	5.2	4.8	5.0	5.8
Fireweed	4.0	4.2	3.4	4.3	5.1	6.0	5.5	5.7	6.8

¹ Averages of 2 determinations.

² Possibly affected by a sampling error.

³ On July 30 and later, no green growth was present.

DISCUSSION

Before considering the data, it should be pointed out that climatically the growing season of 1937 at the Priest River experimental forest was exceptional. The month of May was the driest recorded in 25 years of observation, and both April and June were the wettest on record. July precipitation, although not establishing a record,

⁴ ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 2, 417 pp., illus. Washington, D. C. See p. 72.

was well above average,⁵ and that of August was 31 percent below average.

Plant moisture content undoubtedly plays the major role in the influence of vegetation conditions on rate of fire spread, as was suggested by Hawley's conclusions. Moisture content varies widely, not only seasonally for each species but also on the same date among different species growing side by side (table 1). Especially noteworthy are the data for downy chess and fireweed. The former, which cures very early, was definitely inflammable early in July. Collections of downy chess were discontinued after July 20, because all green color had disappeared and the sample plants were definitely cured. In this state, with only 9 percent of moisture, each gram of this cured material was found capable of producing 3,900 calories to contribute to the spread of fire. Fireweed, in contrast, registered at the end of the season, September 10, a moisture content of 241 percent, which is higher than that of any of the other plants of this group when they were wettest, and on June 20 and 30. With its fuel value of nearly 4,400 calories per gram and moisture content of 241 percent, this plant was capable of producing only 1,300 calories per gram on complete combustion. It is conceivable that, 71 percent of its composition being water, fireweed continues even at the end of the season to exert a retarding effect on the spread of fire.

Observations on test fires in 1935 and 1936 indicated entirely different reactions and burning properties for snowbrush, ninebark, and willows, although the moisture content and calorific values of the plants at the time were similar. As had previously been noted by trained field observers while on actual fires and test burns, the willows exhibited superior fire resistance. At any one time during the 1937 season the differences in water content of snowbrush, ninebark, and willows were not large, never as much as 20 percent (table 1).

This raised the question of the relative tenacity with which plants of different species hold their moisture when subjected to fire. The rate of loss of water might, for instance, be significantly influenced by the fact that part of the water content of green plants is colloiddally bound. Evaporation tests were therefore undertaken specifically to determine the rate of water loss at moderately high temperatures. On September 10, by using a temperature of 170° C. and weighing the samples every half hour, it was found that loss of moisture in the first 30 minutes amounted to 48 percent of total moisture content for freshly picked green leaves of snowbrush, as against 38 percent for similar samples of ninebark and only 32 percent for willow. At a temperature of 170° C., morphological differences of the leaves cannot account for the differences noted in rate of loss of moisture. What, then, are the water-retention forces involved, and how can the energy necessary to overcome these forces be measured? An effort is being made to extend the present study to include an investigation of the nature of the plant juices as a possible partial explanation.

According to the calorific determinations in table 2, only snowbrush and pinegrass varied markedly during the season in total or potential heat energy, the former exhibiting a small gain, the latter a mid-season dip, a recovery, and then a late-season decline. The mid-

⁵ The exceptional wetness of April, June, and July offers a possible explanation of some significant results of the vegetation volume phase of this study. According to the volume measurements, based on oven-dry weight, the plants made 50 percent more total growth in 1937 than in either 1935 or 1936, seasons with rainfall slightly below the mean.

season dip of the pinegrass probably was due to sampling technique, which until after July 30 did not eliminate the possibility of collecting samples from plants that had previously been clipped. The August 10 to September 10 data are believed to indicate the true condition of the species.

The 600-calorie increase noted in snowbrush would seem to be a significant gain in available energy that should cause a marked difference in the fire behavior of this plant as the season progressed. The concurrent decrease of moisture represents a further increase in available heat energy. For example, 1 gm. of the fresh green plant in the field on June 20 contained 0.68 gm. of water and 0.32 gm. of solid material, representing only 1,400 calories. On September 10, 1 gm. of this same plant contained 0.53 gm. of water and 0.47 gm. of solid matter, representing 2,400 calories. The increase of 1,000 calories per gram is possibly significant from the standpoint of the fuel energy/water content relation.

From the information available at present, the quantity of energy necessary to rid living vegetation of its water can be only roughly approximated. On the basis of the thermal capacity of free water, the heat of vaporization, and the specific heat of water vapor, it is estimated that to dissipate the 0.68 gm. of water contained in each gram of green snowbrush on June 20 would require approximately 500 calories. On this same basis, it is estimated that to dissipate the 0.53 gm. of water in the snowbrush on September 10 would require roughly 350 calories. When this decrease from 500 to 350 calories is considered in relation to the concurrent increase of 1,000 calories per gram of green vegetation, it is obvious that the potential rate of spread of fire through snowbrush brush fields should be expected to increase between June 20 and September 10.

As a rule the calorific values of the crude fats are considerably higher, and their ignition temperatures are measurably lower, than those of cellulose. Any significant change in either quantity or nature of these constituents might readily alter the burning properties of the plants. Particularly is this the case for snowbrush, which has been provided by nature with a liberal covering of oleaginous material on the upper surface of the leaf. The crude fat data obtained in these examinations (see table 3) are of interest not only because of the seasonal variations indicated in the quantity of these materials and the relatively large proportion of the total weight that they constitute, but also because of the differences and similarities evidenced between species.

To date, no effort has been made to ascertain the nature of the constituents making up the crude fats. It is conceivable that the continued increase in the calorific value of snowbrush after August 10, despite the crude fat decrease, may be due to a change in the nature of the fats. An increase in the hydrocarbon fraction of the volatile oils would tend to increase calorific values and also introduce an additional danger factor, that of lower ignition temperatures due to increased ease of vaporization. Therefore, it would seem advisable to include this phase in future considerations.

CONCLUSIONS

Quantitative variations in the moisture content of the vegetation on this area in the northern Rocky Mountains appear to exert the

dominant influence on the fire behavior of the plants through the fire season. Moisture content of plants of each of the species studied varied widely, and in some cases wide differences in moisture content were exhibited by neighboring plants of different species. In all the species, a decided decrease in moisture content took place as the season advanced; undoubtedly, this decrease is the principal cause of increased inflammability. As critical fire conditions develop, factors other than total free moisture may gradually assume increased importance. The tenacity with which a given plant holds its free or chemically combined moisture may determine whether it exerts a retarding effect, no effect, or an accelerating effect on the rate of spread of forest fires. As yet insufficient data are at hand to permit any conclusions to be drawn concerning the significance of calorific variations. At present it is impossible to estimate, for example, in any but the most general way, what effect an increase of 600 calories per gram would have on rate of spread.

THE EFFECT OF ALKALINE DUST DILUENTS ON TOXICITY OF ROTENONE-BEARING ROOTS AS DETERMINED BY TESTS WITH HOUSEFLIES¹

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INTRODUCTION

Little information appears to be available regarding the effect of dust diluents on the toxicity of rotenone-bearing plant insecticides. The knowledge that rotenone is deteriorated by alkalies under laboratory conditions has been reported by Jones and Haller³ and Takei, Miyajima, and Ono.⁴ Under field conditions, Walker and Anderson⁵ and others have shown that rotenone-bearing roots mixed with alkaline dust diluents are not as effective as those diluted with neutral or acid carriers. As a result, during the past few years, alkaline diluents such as hydrated lime have been discontinued as carriers for rotenone bearing insecticides, and substitute carriers such as clay, talc, gypsum, sulfur, and many other materials have been recommended. Further knowledge of the losses in toxicity of ground rotenone-containing roots when associated with highly alkaline materials is of major importance for the proper preparation of dust insecticides.

In preliminary reports by the authors⁶ the hydrogen-ion potential of several rotenone-bearing dust insecticides was given. The pH values of these preparations, obtained by the colorimetric, the quinhydrone, and the glass electrode methods, showed that dust diluents range from pH 4.23, in the case of calcium sulfate, to approximately pH 12.55, in the case of hydrated lime. Toxicity tests of these dusts on cabbageworms indicated that a possible relation existed between the hydrogen-ion concentration of the dust mixture and its toxicity to insects. Since a large percentage of the insecticidal dusts are alkaline a detailed study of the effect of alkalinity upon rotenone-bearing dusts was made by testing kerosene extracts of the dust mixtures on houseflies (*Musca domestica* L.).

MATERIALS AND METHODS

The materials used in these studies included a group of dust diluents such as are frequently employed in dust insecticides, and three types of rotenone-bearing plants. In selecting the dust diluents for testing purposes, care was taken to employ representative samples of dusting materials which varied widely in hydrogen-ion potential. The rote-

¹ Received for publication October 20, 1939.

² The writers wish to express their appreciation to Dr. Churchill Eisenhart for the presentation of the statistical data.

³ JONES, HOWARD A., and HALLER, H. L. THE "YELLOW COMPOUNDS" RESULTING FROM THE DECOMPOSITION OF ROTENONE IN SOLUTION. Amer. Chem. Soc. Jour. 53: 2320-2324. 1931.

⁴ TAKEI, SANKICHI, MIYAJIMA, SHIKIRO, and ONO, MINORU. ÜBER ROTENON, DEN WIRKSAMEN BESTANDTEIL DER DERRISWURZEL. XI. ROTENONHARZ. QUANTITATIVE BESTIMMUNG DES ROTENONS UND DES DEGUELINS IM ROTENONHARZ. Deut. Chem. Gesell. Ber. 66: 1826-1833. 1933.

⁵ WALKER, HARRY G., and ANDERSON, LAUREN D. NOTES ON USE OF DERRIS AND PYRETHRUM DUSTS FOR THE CONTROL OF CERTAIN INSECTS ATTACKING CRUCIFEROUS CROPS. Jour. Econ. Ent. 27: 388-393, illus. 1934.

⁶ CLARK, NOBLE, compiler. ALKALINE CARRIERS REDUCE EFFECTIVENESS OF DERRIS FOR CABBAGEWORMS. Wis. Agr. Expt. Sta. Bul. 438: 124. 1937.

———. ALKALINE CARRIERS INJURE ROTENONE. Wis. Agr. Expt. Sta. Bul. 443: 47. 1939.

none-bearing roots which were mixed with these diluents were obtained from three different sources: Derris (*Derris elliptica*) was an import from the Malayan Peninsula; timbo (*Lonchocarpus* sp.) was obtained from Brazil; and barbasco (*Lonchocarpus* sp.) from Peru. Analyses⁷ of these roots showed the derris to contain 5.9 percent of rotenone and 14.72 percent of total ether extractives; the timbo, 5.0 percent of rotenone and 20.3 percent of total ether extractives; and the barbasco, 4.55 percent of rotenone and 16.0 percent of total ether extractives.

The diluents chosen for use in the testing varied in respect to both pH value and chemical nature. The dihydrate calcium sulfate and the powdered magnesium carbonate were chemically pure products. The kaolin, gypsum, talc, and hydrated lime were commercial products, as was the 99½ percent pure dusting sulfur. The tobacco filler, also a commercial product, was derived from byproducts of the tobacco industry.

The dusts employed for testing purposes were prepared by mixing 1 part of the rotenone-bearing root with 9 parts of the diluent. Preparations were made with derris, timbo, and barbasco in the same proportions with each diluent to be tested. The derris samples mixed in this manner contained 0.59 percent of rotenone, and 1.47 percent of total ether extractives. The timbo dust mixtures contained 0.5 percent of rotenone and 2.03 of total ether extractives, while the barbasco mixtures contained 0.45 percent of rotenone and 1.6 of total ether extractives. Replicate tests were conducted with each of the above rotenone-bearing plants.

Before the toxicity tests, the dust mixtures were moistened with distilled water, placed in storage at room temperature, and kept there without light for 7 days. The storage period provided conditions for possible deterioration of the dust samples. Replicate unmoistened samples used as checks were stored in the dark for a similar period.

The hydrogen-ion concentration of the various moistened and unmoistened dust preparations was determined by the glass electrode method. The procedure used in determining the pH values was similar to that employed in determining the pH values of soil samples. A measured amount of the dust mixture was placed in a vial with a definite amount of distilled water. This solution was agitated a fixed number of times and allowed to remain in the vial for 1 hour. The solution was then poured into the testing cup of the glass electrode indicator and the pH value recorded. Three readings were taken and the average was recorded as the pH value of the sample. The readings were made over a 1-, 3-, 5-, and 7-day period. The dusts were then thoroughly dried, repowdered, and passed through a 100-mesh screen before being used for testing purposes.

A modification of Campbell's settling mist method⁸ for testing the relative toxicity of contact insecticides against houseflies was employed in determining the relative toxicity of the various dust preparations. Five-day-old flies were subjected to a settling mist of kerosene containing an extract of the various dusts. The extracts were prepared by steeping 10 gm. of the dust in 20 cc. of oil at room temperature for 24 hours. The "steepate" was then filtered and the filtrate tested immediately against the houseflies. For each test,

⁷ Determined by John Powell & Co.

⁸ ZERMUEHLEN, A. E., and ALLEN, T. C. TESTING FLY SPRAYS. MODIFIED PROCEDURE IN TESTING PETROLEUM BASE INSECTICIDES BY THE SETTLING MIST METHOD. Soap 12 (6): 105-107, illus. 1936.

2.5 cc. of the filtrate was atomized into the bell jar under 25 pounds pressure. The length of the exposure period was 2 minutes. Mortality records were taken over a period of 3 days. Flies subjected to a mist of kerosene and to kerosene extracts of diluents which did not contain rotenone-bearing plant materials were used as checks.

RESULTS

The pH values of a number of carriers for insecticidal dusts are shown in table 1. These determinations indicate that there is considerable variation in reaction among dust carriers. The samples of ground rotenone-bearing roots employed in these tests showed pH values on the acid side, and all samples were quite similar in reaction although the roots were obtained from widely different sources.

Moist rotenone-bearing roots both with and without the diluents were stored in the absence of light for a period of 7 days. The moisture content of these dusts was maintained at approximately 48 percent by weight through daily additions of water during the storage period. The absorption of moisture varied with respect to the hygroscopic properties of the diluent under consideration. There was considerable change in the pH values of the rotenone-bearing roots both with and without the diluent (table 2).

Under laboratory conditions, moist stored dusts commonly produced growth of various micro-organisms. Presumably rapid fermentation processes occurred in these samples as a result of the presence of carbohydrates in the ground roots of the plants. Acidity which accompanied fermentation was due no doubt to the formation of certain organic acids, such as acetic and malic acid. Sugar determinations of derris, timbo, and barbasco roots showed that sufficient carbohydrates were present to account for the rapid fermentation of the stored samples. The hydrogen-ion concentration of sterilized samples of these roots remained unchanged standing, and no apparent growth of micro-organisms occurred during the storage period.

TABLE 1.—*The approximate pH values of various dust diluents and rotenone-bearing roots as determined by glass electrode indicator*

Material ¹	pH	Material ¹	pH
Calcium sulfate.....	4.23	Talc A.....	8.24
Clay A.....	4.30	Talc B.....	8.30
Pyrethrum flowers.....	4.57	Clay J.....	8.36
Kaolin.....	4.76	Copper carbonate.....	8.45
Walnut shell flour.....	5.09	Talc C.....	8.59
Clay B.....	5.13	Bentonite.....	8.64
Tobacco dust.....	5.92	Infusorial earth.....	8.66
Pyrophyllite.....	6.19	Calcium carbonate A.....	8.71
Clay C.....	6.23	Ammonium carbonate.....	8.76
Plaster of paris.....	6.35	Calcium carbonate B.....	9.32
Clay D.....	6.40	Diatomaceous earth.....	9.63
Sulfur (flowers).....	6.46	Magnesium carbonate A.....	9.78
Rare rock phosphate.....	6.50	Gypsum B.....	9.93
Clay E.....	6.54	Sodium carbonate.....	10.90
Gypsum A.....	6.57	Magnesium carbonate B.....	10.93
Clay F.....	6.60	Calcium carbonate C.....	11.10
Clay G.....	6.84	Tobacco filler.....	11.80
Sodium bicarbonate.....	7.50	Hydrated lime.....	12.50
Clay H.....	7.66	Derris.....	6.30
Clay I.....	8.03	Timbo.....	6.55
Distilled water.....	6.45	Barbasco.....	6.57

¹ Symbols represent products of different trade names.

TABLE 2.—*Effect of damp storage for 7 days upon the hydrogen-ion concentration of various rotenone-bearing roots with and without dust diluents*

Dust samples ¹	pH of moist samples after—				
	1 hour	1 day	3 days	5 days	7 days
Derris.....	6.30	6.16	5.65	5.05	4.95
Timbo.....	6.55	5.65	4.23	4.06	4.08
Barbasco.....	0.57	5.91	4.60	4.15	4.12
Derris-calcium sulfate.....	7.50	7.00	7.20	7.64	6.90
Timbo-calcium sulfate.....	7.15	6.85	6.25	5.31	5.20
Barbasco-calcium sulfate.....	6.85	6.50	5.70	4.24	4.12
Derris-kaolin.....	8.00	7.46	7.25	6.32	6.28
Timbo-kaolin.....	8.50	7.78	7.45	6.40	5.81
Barbasco-kaolin.....	8.30	7.30	7.35	6.45	6.15
Derris-sulfur.....	6.60	6.35	5.82	5.38	5.10
Timbo-sulfur.....	6.56	5.77	4.55	4.00	3.82
Barbasco-sulfur.....	6.60	5.81	4.74	4.35	4.22
Derris-gypsum.....	6.00	6.05	5.73	5.48	5.30
Timbo-gypsum.....	6.15	5.76	5.15	4.17	3.98
Barbasco-gypsum.....	6.17	5.90	5.11	4.15	4.05
Derris-talc.....	8.90	8.98	7.60	7.11	7.36
Timbo-talc.....	9.00	8.70	7.35	7.30	6.92
Barbasco-talc.....	8.88	8.20	7.32	6.82	6.30
Derris-magnesium carbonate.....	10.20	10.05	10.00	9.80	9.52
Timbo-magnesium carbonate.....	10.10	9.80	9.78	9.45	9.23
Barbasco-magnesium carbonate.....	10.00	9.75	9.50	9.30	9.15
Derris-tobacco filler.....	11.85	11.85	11.70	11.76	11.69
Timbo-tobacco filler.....	11.83	11.81	11.72	11.75	11.78
Barbasco-tobacco filler.....	11.88	11.75	11.75	11.73	11.72
Derris-hydrated lime.....	12.50	12.50	12.50	12.50	12.47
Timbo-hydrated lime.....	12.52	12.50	12.48	12.42	12.50
Barbasco-hydrated lime.....	12.55	12.51	12.49	12.48	12.49

¹ 9 parts of diluent to 1 part of rotenone-bearing roots.

Fermentation and consequent formation of acids did not occur in the highly alkaline samples. This finding was confirmed in part by observations for perceptible growth of microflora and by pH determinations, which showed no change during the storage period. The acid dust mixtures, however, were a favorable medium for the development of micro-organisms, and in these samples there was a tendency for acids to form. From these results it appears that the acid dust preparations in which greater activity of micro-organisms occurred, tended to retain their toxicity to houseflies. This is contrary to the reports of some workers that micro-organisms present in stored derris solutions are responsible for the deterioration of the toxic ingredients.

In some instances, the final dust mixtures (tables 1, 2, and 3) had pH values greater than those of either the rotenone-bearing material or the diluent. This may be due to some reaction between the diluent and the ground root when they are in an aqueous medium. In such reactions more hydroxyl-ions may be liberated, giving rise to a more alkaline reading.

To determine approximately the minimum quantity of moisture that would bring about a deterioration in the toxicity of the insecticides, further tests were made. Four representative dust samples were dried, three replicates of each sample weighed, placed in a constant temperature-humidity chamber at 73° F. and 61 percent relative humidity, and kept there for 7 days. The samples were then removed from the chamber and reweighed to determine the amount of moisture absorbed. The moisture content of the different samples ranged from 0.3 to 9.7 percent. When oil filtrates of these samples were tested on houseflies, the alkaline samples showed a deterioration quite similar to that of the alkaline samples in table 3, in which the samples contained approximately 48 percent of moisture.

TABLE 3.—Percentage kill of houseflies by kerosene extracts of derris, timbo, and barbasco dust mixtures, when tested following a 7-day period in damp storage

Rotenone-bearing dust ¹	pH	Tests made	Flies tested	Flies killed		Average kill
		Number	Number	Number	Percent	Percent
Derris-sulfur	6.60	6	274	273	99.6	99.2
Timbo-sulfur	6.56	6	257	254	98.8	
Barbasco-sulfur	6.60	6	262	260	99.2	
Derris-gypsum	6.00	6	239	239	100.0	97.4
Timbo-gypsum	6.15	6	265	260	98.1	
Barbasco-gypsum	6.17	6	261	246	94.2	
Derris-buffer ²	6.57	6	285	278	97.6	96.0
Timbo-buffer ²	6.57	6	268	246	91.8	
Barbasco-buffer ²	6.57	6	273	269	98.5	
Derris-calcium sulfate	7.50	6	249	212	85.1	90.2
Timbo-calcium sulfate	7.15	6	240	225	94.1	
Barbasco-calcium sulfate	6.85	6	269	246	91.4	
Derris-talc	8.90	6	244	59	24.1	35.0
Timbo-talc	9.00	6	252	117	46.4	
Barbasco-talc	8.88	6	229	78	34.1	
Derris-magnesium carbonate	10.20	6	233	46	19.7	24.8
Timbo-magnesium carbonate	10.10	6	262	93	35.5	
Barbasco-magnesium carbonate	10.00	6	222	39	17.6	
Derris-tobacco filler	11.85	6	255	16	6.3	27.0
Timbo-tobacco filler	11.83	6	252	99	39.3	
Barbasco-tobacco filler	11.88	6	247	89	36.0	
Derris-hydrated lime	12.50	6	275	18	6.5	25.2
Timbo-hydrated lime	12.52	6	287	118	41.1	
Barbasco-hydrated lime	12.55	6	269	73	27.1	
Derris-hydrated lime ³	12.50	8	321	302	94.1	93.2
Timbo-hydrated lime ³	12.52	8	343	324	94.4	
Barbasco-hydrated lime ³	12.55	8	339	309	91.1	
Check	-----	17	644	22	3.4	3.4

¹ 9 parts of diluent to 1 part of rotenone-bearing roots.² The buffer was made from M/20 solution of potassium acid phthalate and M/15 solution of potassium acid phosphate which were adjusted to pH 4.5.³ Unmoistened sample.

From the results of the relative toxicity tests of kerosene extracts of the various dust samples shown in table 3, it is apparent that the alkaline dust mixtures talc, magnesium carbonate, tobacco filler, and hydrated lime when moistened cause significant losses in toxicity of rotenone-bearing plants. The fact that timbo retains much of its toxicity to houseflies in the higher alkaline ranges may be explained by the presence of a larger percentage of resinous materials in the plant sample. This would indicate that rotenone is the major ingredient that is deteriorated by the alkalinity of the dust mixtures. Marked deterioration was evident in all samples more alkaline than pH 7.16.

In preliminary tests with identical dust samples used on cabbage-worms, the addition of sulfur had improved the toxicity of the rotenone-bearing dusts. Consequently, sulfur was added to the highly alkaline diluents at the rate of 1 pound of the diluent to 9 pounds of sulfur. This mixture was maintained at approximately 48 percent moisture and stored as previously described. Although these dust samples remained alkaline after the addition of sulfur, they showed practically no loss in toxicity when tested on houseflies (table 4). Oil filtrates of a dust containing only sulfur and the diluent exhibited almost no toxicity to houseflies.

The entries in tables 3 and 4 that show the percentage of flies killed (columns 6 and 7, respectively) are the weighted means of the replications in each test. By the substitution of letters for degrees of significance, as in tables 5 and 6, a comparison can be made of any insecticidal dust with another in the same row of the table. Where the letters are identical, the two observed "percent killed" were not

sufficiently different to be judged significant at the 5-percent level of significance. However, if different letters are associated with them, it is implied that the difference is significant. This interpretation of letters applies only when reading across a row, and these letters cannot be used for comparisons within a column of the table. Where two letters occur together, the observed percent kill of this dust lay between the values found for the dusts having these letters as indices and was not significantly different from either. The dusts corresponding to the italic letters did not give consistent percent kills in their subsamples, so that the letter by which they are designated may be in error in one direction of the other. In the case of the entries corresponding to talc in table 5, however, there can be no doubt that the talc dusts give significantly lower percent kills than those to the left of them in the table, since there is a strong line of demarcation at this point in the pH scale, as can be seen in table 3.

TABLE 4.—Percentage kill of houseflies by kerosene extracts of *Derris*, *Timbo*, and *Barbasco* dust mixtures containing sulfur, when tested after a 7-day period in damp storage

Rotenone-bearing dust ¹		pH	Tests made	Flies tested	Flies killed		Average kill
Root	Diluent ²						
			<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Percent</i>	<i>Percent</i>
Derris.....	Magnesium carbonate	9.00	6	252	237	94.0	96.1
Timbo.....	do.....	9.07	6	238	229	96.2	
Barbasco.....	do.....	9.17	6	241	237	98.3	
Derris.....	Tobacco filler	9.03	6	277	276	99.6	99.2
Timbo.....	do.....	9.33	6	270	268	99.2	
Barbasco.....	do.....	9.51	6	273	270	98.9	
Derris.....	Hydrated lime	11.23	6	275	269	97.8	95.3
Timbo.....	do.....	11.46	6	264	241	91.2	
Barbasco.....	do.....	11.17	6	276	268	97.1	
	Sulfur		8	334	12	3.5	3.5
	Check		11	354	11	3.1	3.1

¹ 9 parts of diluent to 1 part of rotenone-bearing roots.

² Contained sulfur at rate of 9 parts to 1 of diluent by weight.

The letter "g" has been used here in connection with the check to emphasize the great difference between the check and the other entries in the table and the similarity of the behavior of the check in all the experiments.

TABLE 5.—Statistical significance of data in table 3 ¹

Rotenone-bearing root	Significance for diluent indicated ¹									Check
	Sulfur	Gypsum	Buffer	Calcium sulfate	Talc	Magnesium carbonate	Tobacco filler	Hydrated lime	Unmoistened hydrated lime	
Derris.....	a	a	b	d	e	e	f	fg	c	g
Timbo.....	a	a	b	b	c	d	cd	c	b	g
Barbasco.....	a	b	a	c	d	f	d	e	c	g

¹ Computed from original replications from which the data in table 3 were obtained by using the χ^2 table for 1 degree of freedom and the 5-percent level of significance:

$$\chi^2 = \frac{[P_1 - P_2]^2}{P[1 - P]\left\{\frac{1}{N_1} + \frac{1}{N_2}\right\}}, \text{ with } P = \frac{N_1 P_1 + N_2 P_2}{N_1 + N_2}.$$

¹ See text for significance of letters.

TABLE 6.—*Statistical significance of data in table 4*¹

Rotenone-bearing root	Significance for diluent indicated ²			Sulfur	Check
	Magnesium carbonate	Tobacco filler	Hydrated lime		
Derris	b	a	a	g	g
Timbo	b	a	c	g	g
Barbasco	a	a	a	g	g

¹ See footnote 1, table 5.² See text for significance of letters.

SUMMARY

Diluents used in the preparation of rotenone-bearing dusts show a wide range in hydrogen-ion potential which is of considerable importance in the utilization of such materials as plant insecticides.

Rotenone-bearing dusts prepared from highly alkaline diluents and kept in damp storage in the absence of light for a period of 7 days, exhibited little or no change in pH, but showed considerable loss in toxicity when used in kerosene extracts in tests with houseflies.

Parallel acid dust samples kept under identical storage conditions retained their toxicity to the housefly. Dry or unmoistened alkaline and acid samples remained unchanged in this respect.

The addition of sulfur to the alkaline dust mixtures prevented deterioration of the rotenone-bearing dust mixtures under the conditions of this experiment.

THE REPRODUCTIVE EFFICIENCY OF THE ALBINO RAT UNDER DIFFERENT BREEDING CONDITIONS¹

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INTRODUCTION AND REVIEW OF LITERATURE

The problem of determining the most efficient breeding age for animals is an old one, but quantitative data on the subject have been obtained only comparatively recently. In 1909 Mumford started a thorough study at the Missouri Experiment Station on the growth and reproduction of swine under various regimes, which has been continued to the present time. McKenzie (11)² summarized the results previously reported in detail by the other Missouri workers. Table 1 presents averages from their data. The litters from the sows which were bred early and those which were bred at a normal age were approximately equal in weight and in number of young. The group of sows that were bred late, on the other hand, had much smaller litters. The sows that were bred early (while they were still in the growing period) were considerably retarded in growth by the added demands of raising their first litters, but they continued to grow twice as long as did the others. They therefore reached about the same ultimate size as the sows of the other groups. Carmichael and Rice (3) carried out a somewhat different experiment, also with swine, and in general confirmed the Missouri results as far as the growth trends were concerned.

TABLE 1.—*The effect of age at which sows were bred upon the weight, number, and and vitality of their young*¹

Period of sow's life when bred	Age of sows	Birth weight of young	Total litter weights at birth	Young per litter at birth	Young alive after 12 hours	Young weaned	Mature weight of sows
	<i>Days</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Pounds</i>
Early in life	218.1	2.30	20.01	8.68	6.62	4.42	415.3
At normal age	478.6	2.38	19.86	8.36	7.72	6.36	401.0
Late in life	837.6	2.85	14.91	5.62	5.06	4.00	384.4

¹ Data from Missouri Agricultural Experiment Station Research Bulletin 118, tables 1 and 3 (for mature weight of sows).

With dairy cattle, early breeding has the special advantage of bringing the heifer into milk production sooner, in addition to the value of the calf produced. The Missouri workers, Eckles and Sweet (6), demonstrated, however, that lactation markedly retards growth, as measured by weight and skeletal size. They found that if lactation occurs early the mature size of the animal will be affected.

² Received for publication December 11, 1939. These studies were supported in part by a grant from Bankhead-Jones project 14, and in part by a grant from the Rockefeller Grant for Research in Longevity.

³ Italic numbers in parentheses refer to Literature Cited, p. 852.

For sheep, Briggs (2) found that ewes bred at their first season had a slower growth rate than ewes bred later, but that they reached the same ultimate size. They also produced rather more than half a lamb extra, but more dental defects became apparent as they grew older.

The work of Bogart et al. (1) with rats has shown quite definitely that pregnancy exerts a strong stimulus on growth while lactation as definitely retards growth. Slonaker (14) and Cole and Hart (4) have obtained similar results using somewhat different methods. It may be noted here that none of the work quoted has shown the existence of the pregnancy stimulus in the domestic animals. This may possibly be owing to the fact that other conditions in these experiments were not kept as uniform as they have been in the rat experiments. Another possibility is that the pregnancy stimulus can only make itself felt when the potentiality for growth is still fairly great. The rat seems to be peculiar in that it retains growth potentialities until quite late in life (10). The writers have obtained the pregnancy growth stimulus even in rats bred at 280 days (the normal breeding age is about 100 days, and puberty about 65 days).

Other factors of importance in the over-all efficiency of breeding animals are the number of young produced at a time and the interval between their production. This has rarely been investigated in domestic animals as it means that the animals have to be kept for their whole lifetime whether they are economically efficient or not. Most of the reports which have been made on the subject have grouped animals, such as early, normal, or late breeders, according to their behavior. This method of grouping does not tell which is the best regime to follow since an individual falls into its class because it is unable to do otherwise. It tells what happens as a rule, but not what should be encouraged to happen in order that the best results may be obtained.

The writers made a study of the effects of various reproductive regimes on albino rats to determine which one should be followed in order to get the optimum breeding results. Rats were used because they have a much shorter and more prolific life than the domestic animals. The data in the present paper are a part of the information collected in the study.

EXPERIMENTAL PROCEDURE

Four groups of 50 female albino rats each were used to test the differences in the over-all reproductive efficiency of the various breeding regimes. The rats were all born about the same time. The division into groups was made at random, except that litter mates were put into different groups. The groups were bred as follows:

Group 1 was bred as early as the rats would breed and is referred to as the bred-early group.

Group 2 was bred at the normal breeding age (100 days), and is referred to as the bred-normal group.

Group 3 consisted of rats whose breeding was deferred until the animals were 280 days old. This group is referred to as bred late.

Group 4, like group 2, was bred at the normal breeding age, but the rats were not allowed to suckle their young and were bred as soon as possible after parturition. This group is referred to as young killed.

The rats were kept in cages containing five females each. They had constant access to the usual stock diet for the station rat colony and water. Also each cage always contained one male. These males were

shifted every week from one cage to the next within the same group. Whenever a male was found to be defective in any way he was immediately replaced. All males were replaced by young males when they were a year old.

When the females became pregnant, as detected by palpation, they were isolated, and with the exception of group 4 were allowed to rear their young to 3 weeks of age. At this time the young were weaned and the females returned to their respective breeding cages. If the young did not survive until weaning time, the females were nevertheless kept isolated for 3 weeks following the date of parturition. The females of group 4, however, were returned to their breeding cages within 1 day after parturition. Complete records were made of the number, weight, and sex of all young at birth and at weaning.

The data recorded here refer only to litters born on or before the rats were 671 days old, since subsequent data were rendered useless by the accidental killing of 60 animals. Abortions, when they were seen, were charged against the rat as the production of a zero litter since they represent an attempt at reproduction and the loss of so much time and efficiency. When all the young were dead at birth, this also was charged against the rat as a zero litter for the same reason. Other young born dead were not counted in this work as they do not represent productive reproduction. Females which were sterile from the outset of the experiment were included in the groups; there was no way of determining whether the sterility was caused by the reproductive regime.

Examination of tables 2 and 3 shows that the reproductive efficiency of group 1, as measured by the number of litters and the number and weight of living young at birth and at weaning, was somewhat less than that of the animals bred at a normal age. The difference amounts to 9.1 percent for the number born in favor of group 2. This is in spite of the earlier start by the rats that were bred early. Group 1 has a slightly better weaning percentage, but the number weaned is still 8.8 percent greater for group 2. From the data there appears to be no advantage in early breeding, but slightly the reverse.

TABLE 2.—*The effect of age at which female rats were bred, and of killing their offspring at birth, upon the weight, number, and vitality of their young*

Group No. ¹	Litters	Young alive at birth	Weight of young alive at birth	Young alive at weaning	Weight of young alive at weaning	Average maximum weights of nonpreg- nant females
	Numbers	Number	Grams	Number	Grams	Grams
1, bred early	426	2,487	14,374	1,974	62,773	310.3
2, bred at normal age	436	2,714	15,731	2,148	68,618	308.6
3, bred late	255	1,257	7,447	898	30,224	296.3
4, young killed	660	3,984	22,743			323.9

¹ See text for explanation of groups; there were 50 rats in each group.

The females of group 3 had by far the smallest number of litters and the smallest sized litters. In accordance with the usual finding that the weight of the young varies inversely with the number in the litter, individual young of this group averaged slightly heavier than those of other groups. However, it should be noted that the young of group 1

were not larger than those of group 2 although their litters were appreciably smaller than the litters of the latter.

TABLE 3.—*The effect of age at which female rats were bred, and of the killing of their offspring, upon the weight, number, and vitality of their young, the results being expressed on a per-rat basis*¹

Group No. ²	Litters per rat	Young per litter at birth	Young per rat	Weight of young at birth	Weight of young at weaning	Young weaned
	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Grams</i>	<i>Grams</i>	<i>Percent</i>
1, bred early.....	8.5±0.35	5.80±0.07	49.7±2.11	5.78	31.8±0.09	79.4
2, bred at normal age.....	8.7±0.29	6.22±0.08	54.3±1.86	5.80	31.9±0.10	79.1
3, bred late.....	5.1±0.23	4.90±0.11	25.1±1.29	5.92	33.7±0.15	71.4
4, young killed.....	13.2±0.50	6.04±0.07	79.7±3.02	5.71		

¹ Probable errors shown.

² See text for explanation of groups; there were 50 rats in each group.

Statistical analysis shows that in the numbers of litters per rat the difference between group 1 and 2 is not significant. Groups 3 and 4, however, are significantly different. In the number of young per litter at birth the animals of group 1 are significantly lower than those in group 2, but those in group 4 are not. The rats in group 3 are significantly lower. In the number of young per rat, group 1 is not significantly lower than group 2. The difference is twice the probable error. Groups 3 and 4 differ significantly from group 2. No analysis is possible of the weights at birth as these figures were obtained by dividing the sums of the weights of the whole litters by the total number born. The difference in the weight of young at weaning is not significant between the groups 1 and 2, while the increased weight of young in group 3 is very significant. This is associated with the smaller litters and the high mortality which probably gave the young more milk each and assisted in the culling of many of the unfit. The percentage of young weaned is a percentage of the whole, and not a litter by litter average, so these figures are not analyzed.

Group 4 was able to have a considerably larger number of litters since the rats were rebred immediately after parturition instead of 3 weeks later. Their litters averaged slightly less in number of young, but they produced 47 percent more young than those in group 2. This was despite the fact that two of them produced no litters. This very intense production was not an undue drain on the females, on the contrary they made the best growth of all the groups considered in this paper. This is shown in table 2 which reports the average maximum weights of nonpregnant animals in each group. Lactation is a far greater drain on the mother than reproduction, even of the most intense kind. Indeed, pregnancy must be regarded as a stimulus, or more probably as the means of removal of an inhibitor for growth. This inhibitor is believed to be estradiol, since virgin rats attain a much lower ultimate weight than bred rats, while ovariectomized rats attain the largest size.

It may be noted in passing that group 4 had considerably larger litters than group 2 early in life, but that the litter size dropped off markedly so that in later life their litter size was less than that of group 2.

In order to compare the reproductive efficiencies of the different groups in more detail, the reproductive patterns are given in table 4.

TABLE 4.—*The effect of age at which female rats were bred, and of killing their offspring at birth, upon their reproductive patterns*

Group No. ¹	Sterile rats	Rats having only 1 litter	Rats not reproducing after 1 year of age	Litters conceived within 8 days after rats were rebred
	Number	Number	Number	Percent
1. Bred early	0	4	13	65.8
2. Bred at normal age	0	2	7	69.6
3. Bred late	2	2	6	62.2
4. Young killed	2	0	8	72.1

¹ See text for explanation of groups; there were 50 rats in each group.

A study of table 4 shows that the lower reproductive efficiency of the group 1 in comparison with group 2 involves three factors: (1) A larger number of the rats in group 1 had ceased to reproduce by the time they were 1 year old; (2) a smaller percentage of the litters were conceived within 8 days after parturition, that is, a larger percentage of the litters were irregular in conception, thus spacing the litters further apart; and (3) the litters were smaller (table 3).

These factors more than compensate for the earlier start of the rats in group 1. Group 3 had a decidedly lower over-all reproductive efficiency because (1) it had a great handicap in not starting breeding as soon as the others, (2) it had the most delays, that is, the most irregular litters, and (3) it had considerably smaller litters (table 3).

Group 4 owes its efficiency to the fact that these rats did not lose the 3 weeks required to rear their young. Also, the females were in better condition and therefore had more litters which were more regularly conceived.

The group 3 rats had more difficulty in rearing their young than did the other groups so that the percentage weaned is considerably lower than in the other groups. This is in spite of the fact that they had smaller litters to rear, a fact which is reflected in the greater average weaning weights in this group, though the average increase in weight at weaning does not outweigh the greater loss of young. Group 3 lost 70 litters entirely during lactation as compared with 46 litters for group 1, and 57 for group 2. If these figures are taken at their face value it would indicate that early breeding is decidedly favorable for the establishment of good lactation. Table 3 does not reveal, however, a higher level of lactation for the individual rats of group 1.

DISCUSSION

In general the data are in agreement with the results of other workers. The results indicate perhaps that early breeding has some deleterious effects but that these are not great. They do not suggest that early breeding should be resorted to as a breeding practice, but naturally in work of this sort it is necessary to determine the effects from one species to another. The lifetime efficiency in groups studied as a whole is essential for such work, and culling immediately destroys its value. If the animals are not in the best of condition and if the breeding occurs very early, the practice will usually result in somewhat inferior young and slightly stunted females. This would suggest a compromise in practice, where conditions are usually not optimum. Breeding late is definitely uneconomical.

The data on the rats in group 4 show a relatively smaller litter size as compared with those classed as normal than that found by Slonaker (14, 16). But his numbers were meager, and the conditions of the experiment were not quite the same as those in the present experiment. The growth of the females used in the present study is in partial disagreement with the data of Cole and Hart (4), but their animals were subjected to other conditions which may have affected the results. Slonaker's rats were divided between stationary and rotary cages which altered the reproductive regime somewhat, and the rats of Cole and Hart were injected with mare gonadotropic hormone.

The rats used by the present writers did not show any significant variation in the number of mammary tumors which occurred before they were 22 months of age. Two of the females in group 4 and one in each of the other groups had these tumors.

The data show that rats which were not subjected to lactation were able to carry through a much larger number of gestations. It has been repeatedly shown that lactation is a much more severe tax on the animal than is gestation. The increased demands made by lactation are reflected by the increased food consumption (5, 12, 13, 15); by the negative mineral balance (7, 8), and by the change in composition of the body tissues (9).

SUMMARY

The reproductive efficiency of the albino rat under various reproductive regimes was investigated. Four groups of 50 rats each were studied until they were 22 months of age. A total of 1,777 litters were studied. The over-all reproductive efficiency of group 1 (early bred) as measured by the total number and weight of young produced was somewhat less than that of the rats bred at a normal age, and the reproductive efficiency of the group in which breeding was delayed until 9 months of age was very much lower than that of any of the other groups. They also experienced much more difficulty in lactation.

The animals which were not allowed to suckle their young and which were rebred immediately after parturition, produced many more young than did the groups which were subjected to lactation.

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